

## Mycobacterial cell wall biosynthesis

Abrahams, Katherine A; Besra, Gurdyal S

DOI:

[10.1017/S0031182016002377](https://doi.org/10.1017/S0031182016002377)

License:

Creative Commons: Attribution (CC BY)

*Document Version*

Publisher's PDF, also known as Version of record

*Citation for published version (Harvard):*

Abrahams, KA & Besra, GS 2016, 'Mycobacterial cell wall biosynthesis: a multifaceted antibiotic target', *Parasitology*. <https://doi.org/10.1017/S0031182016002377>

[Link to publication on Research at Birmingham portal](#)

### **Publisher Rights Statement:**

COPYRIGHT: © Cambridge University Press 2016

This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence

(<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution, and reproduction in any medium, provided the original work is properly cited.

### **General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

### **Take down policy**

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

# Mycobacterial cell wall biosynthesis: a multifaceted antibiotic target

KATHERINE A. ABRAHAMS and GURDYAL S. BESRA\*

*Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK*

(Received 7 September 2016; revised 2 November 2016; accepted 8 November 2016)

## SUMMARY

*Mycobacterium tuberculosis* (*Mtb*), the etiological agent of tuberculosis (TB), is recognized as a global health emergency as promoted by the World Health Organization. Over 1 million deaths *per* year, along with the emergence of multi- and extensively-drug resistant strains of *Mtb*, have triggered intensive research into the pathogenicity and biochemistry of this microorganism, guiding the development of anti-TB chemotherapeutic agents. The essential mycobacterial cell wall, sharing some common features with all bacteria, represents an apparent ‘Achilles heel’ that has been targeted by TB chemotherapy since the advent of TB treatment. This complex structure composed of three distinct layers, peptidoglycan, arabinogalactan and mycolic acids, is vital in supporting cell growth, virulence and providing a barrier to antibiotics. The fundamental nature of cell wall synthesis and assembly has rendered the mycobacterial cell wall as the most widely exploited target of anti-TB drugs. This review provides an overview of the biosynthesis of the prominent cell wall components, highlighting the inhibitory mechanisms of existing clinical drugs and illustrating the potential of other unexploited enzymes as future drug targets.

**Key words:** tuberculosis, cell wall, peptidoglycan, arabinogalactan, mycolic acids, antibiotics.

## INTRODUCTION

*Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis (TB), is regarded as the world’s most successful pathogen (Hingley-Wilson *et al.* 2003). Responsible for an estimated 1·4 million deaths and 10·4 million new cases of TB, including 480 000 new cases of multi-drug resistant (MDR)-TB in 2015 (World Health Organization, 2016), *Mtb* remains a global health emergency as declared by the World Health Organization (WHO) (World Health Organization, 2014). New chemotherapeutic agents to complement or replace existing front-line treatment regimens are urgently required to reduce treatment time (currently 6-month course) and to combat the increasing threat by this microorganism.

The distinguishing feature of mycobacteria, the complex cell wall, is a well-recognized drug target. The cell wall is common to all bacteria, both Gram-positive and Gram-negative, but can have vast differences in terms of the biochemical and structural features. Over the past decade, extensive research into cell wall assembly, aided by whole-genome sequencing, has led to an increased understanding of mycobacterial cell wall biosynthesis. This has promoted further exploration into the discovery and development of chemotherapeutic agents (from an

enzymatic and phenotypic perspective) directed against the synthesis of this unique macromolecule structure in *Mtb*. The *Mtb* cell envelope is an expansive structure and is summarized in Fig. 1. The inner membrane phospholipid bilayer contains glycolipids that extend into the periplasmic space. The essential core cell wall structure is composed of three main components: a cross-linked polymer of peptidoglycan, a highly branched arabinogalactan polysaccharide, and long-chain mycolic acids. Intercalated into the mycolate layer are solvent-extractable lipids including non-covalently linked glycopospholipids and inert waxes, forming the outer membrane. The capsule forms the outermost layer and is mainly composed of proteins and polysaccharides. The lipid- and carbohydrate-rich layers of the cell wall serve not only as a permeability barrier, providing protection against hydrophilic compounds, but also are critical in pathogenesis and survival. It is these traits that make the biosynthesis and assembly of the cell wall components attractive drug targets. This review focuses on the synthesis of the key cell wall components, highlighting previously validated targets and the ongoing drug discovery efforts to inhibit other essential enzymes in mycobacterial cell wall biosynthesis.

## PEPTIDOGLYCAN

Peptidoglycan is a major component of the cell wall of both Gram-positive and Gram-negative bacteria (Vollmer *et al.* 2008). It is a polymer of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid

\* Corresponding author: Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK. E-mail: [g.besra@bham.ac.uk](mailto:g.besra@bham.ac.uk)

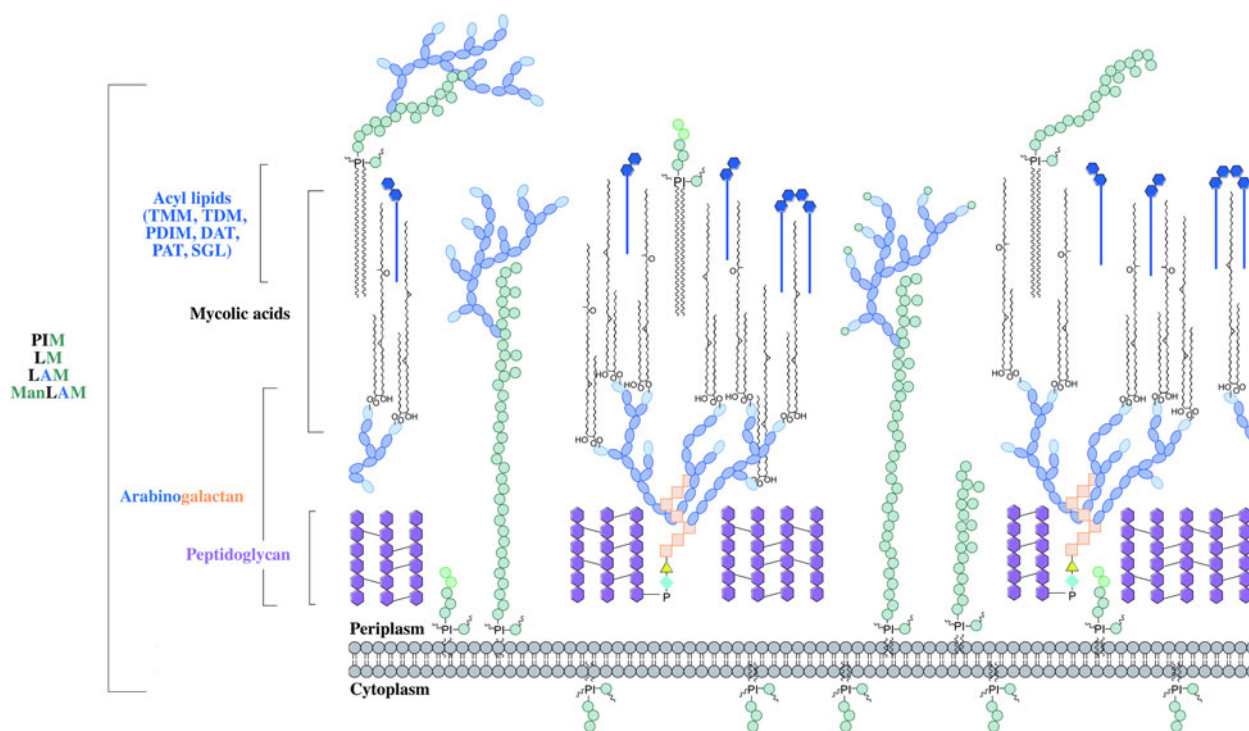


Fig. 1. The mycobacterial cell wall. A schematic representation of the mycobacterial cell wall, depicting the prominent features, including the glycolipids (PIMs, phosphatidyl-*myo*-inositol mannosides; LM, lipomannan; LAM, lipoarabinomannan; ManLAM, mannosylated lipoarabinomannan), peptidoglycan, arabinogalactan and mycolic acids. Intercalated into the mycolate layer are the acyl lipids (including TMM, trehalose monomycolate; TDM, trehalose dimycolate; DAT, diacyltrehalose; PAT, polyacyltrehalose; PDIM, phthiocerol dimycocerosate; SGL, sulfoglycolipid). The capsular material is not illustrated.

residues via  $\beta(1 \rightarrow 4)$  linkages with side chains of amino acids cross-linked by transpeptide bridges (Brennan and Nikaido, 1995). Mycobacterial peptidoglycan has a number of unique features that diversifies the cell wall from the typical structure including *N*-glycolyl- and *N*-acetyl-muramic acid residues (Mahapatra *et al.* 2005a), amidation of the carboxylic acids in the peptide stems (Mahapatra *et al.* 2005b) and additional glycine or serine residues (Vollmer *et al.* 2008). The function of peptidoglycan is not only to provide shape and rigidity, but it is responsible for counteracting turgor pressure and hence it is essential for growth and survival (Vollmer *et al.* 2008). Peptidoglycan is unique to bacterial cells, and it is this property that has led to numerous enzymes involved in its synthesis to be targeted by potent antibiotics, with others representing attractive targets in the development of future antibiotics.

#### PEPTIDOGLYCAN BIOSYNTHESIS

The biosynthesis of peptidoglycan is summarized in Fig. 2. The first committed step is the generation of uridine diphosphate-*N*-acetylglucosamine (UDP-GlcNAc). This is catalysed by the acetyltransferase and uridylyltransferase activities of GlmU (Zhang *et al.* 2009), where first the acetyl group from

acetyl-CoA is transferred to glucosamine-1-phosphate (GlcN-1-P) to produce *N*-acetylglucosamine-1-phosphate (GlcNAc-1-P). Secondly, uridine-5'-monophosphate from UTP is transferred to GlcNAc-1-P to yield UDP-GlcNAc (Zhang *et al.* 2009). The abundance of GlcNAc-1-P in eukaryotes (Mio *et al.* 1998) and the functional similarity of the GlmU uridylyltransferase with human enzymes (Peneff *et al.* 2001) makes this domain an unsuitable drug target (Rani and Khan, 2016). However, the absence of GlcN-1-P from humans makes the acetyltransferase domain a potential target (Mio *et al.* 1998). Efforts to identify inhibitors of this domain are underway (Tran *et al.* 2013). A substrate analogue of GlcN-1-P has been designed and exhibits inhibitory effect against GlmU, providing a candidate for further optimization (Li *et al.* 2011).

The next step involves the generation of the UDP-*N*-acetylmuramic acid (UDP-MurNAc)-pentapeptide, which is synthesized in a sequential pathway catalysed by the Mur ligases A–F (Barreteau *et al.* 2008), whereby most of the *Mtb* genes have been found through homology. MurA, a UDP-*N*-acetylglucosamine 1-carboxyvinyltransferase, and MurB, a UDP-*N*-acetylenolpyruvoylglucosamine reductase, are involved in generating UDP-MurNAc from UDP-GlcNAc, by first the addition of the enolpyruvyl moiety of PEP, followed by reduction to a lactoyl ether moiety via NADPH. At this point, NamH, a

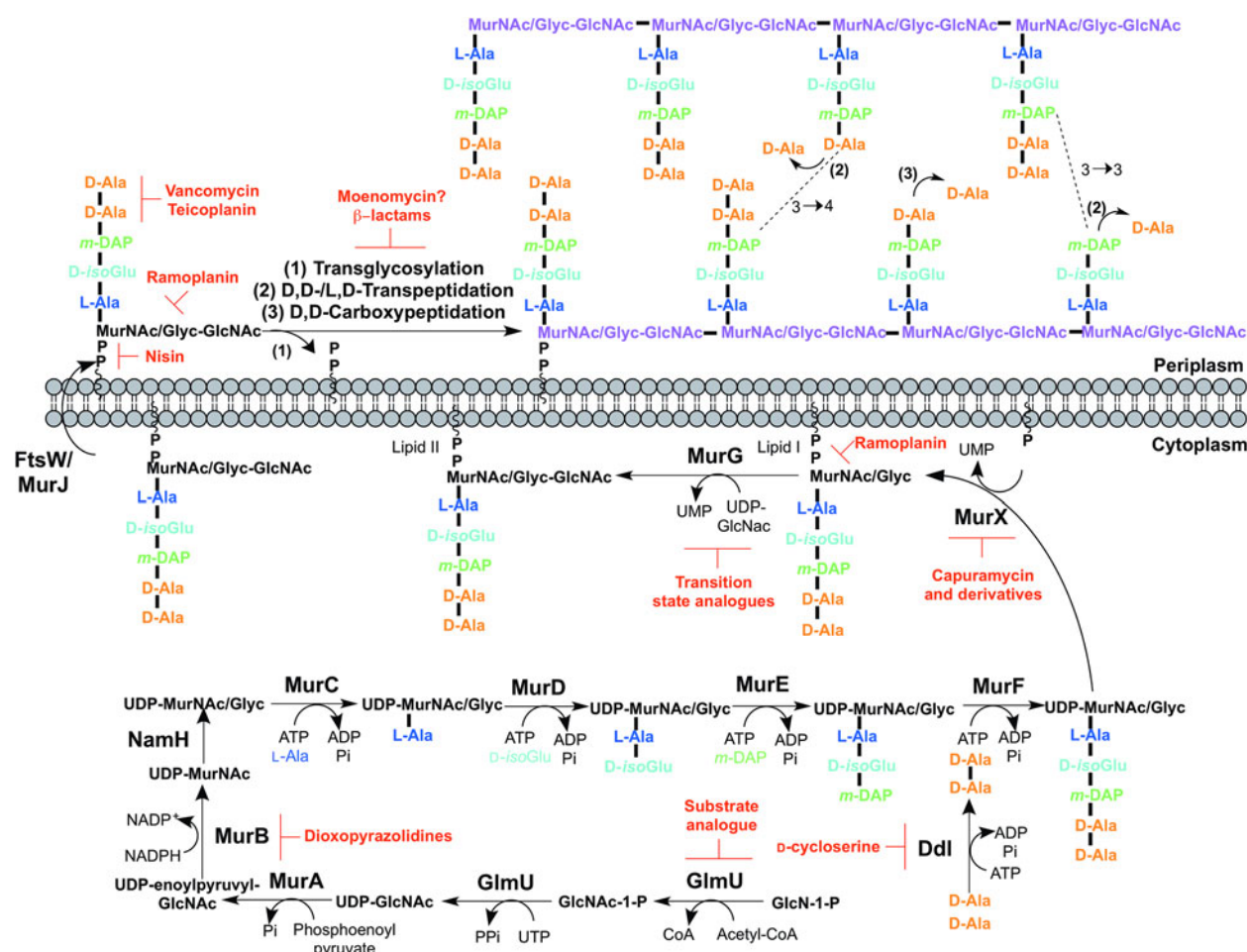


Fig. 2. Inhibitors targeting peptidoglycan biosynthesis. The roles of the key enzymes involved in peptidoglycan biosynthesis are illustrated. Reported inhibitors are shown in red.

UDP-*N*-acetylmuramic acid hydroxylase, hydroxylates UDP-MurNAc to UDP-*N*-glycolylmuramic acid (UDP-MurNGlyc), providing both types of UDP-muramyl substrates; *Mtb* cell walls are dominated by the latter (Mahapatra *et al.* 2005a). This structural modification is unique to mycobacteria (and closely related genera) and is considered to increase the intrinsic strength of peptidoglycan, by potentially alleviating susceptibility to lysozyme and providing sites for additional hydrogen bonding (Raymond *et al.* 2005). Inhibitors of *Mtb* MurA and MurB are yet to be discovered. Whilst the natural product, broad spectrum antibiotic, fosfomycin, targets Gram-negative MurA, the critical residue for inhibition is absent in *Mtb*, providing intrinsic resistance against this antibiotic (Kim *et al.* 1996). Consequently, an inhibitor with a new mode of action is required to target *Mtb* MurA. A limited number of inhibitors have been reported against MurB. Molecular dynamics and docking studies of existing MurB inhibitors (3,5-dioxopyrazolidine derivatives) onto the *Mtb* MurB structure reveal the potential potent activity of these compounds, which can be used to guide future structure-based drug design (Kumar *et al.* 2011). Inhibitors of NamH have not been documented; *namH* is not essential in

*Mycobacterium smegmatis*, and therefore is not conducive to a characteristic target property. However, gene deletion results in a strain hypersusceptible to β-lactam antibiotics and lysozyme and therefore inhibitors of NamH could potentiate the effect of β-lactams (Raymond *et al.* 2005).

The pentapeptide chain is incorporated onto the UDP-MurNAc/Glyc substrates by the successive addition of amino acid residues L-alanine, D-isoglutamate, meso-diaminopimelate (*m*-DAP) and D-alanyl-D-alanine [generated by the D-Ala:D-Ala ligase (Ddl)] by the ATP-dependent Mur ligases C-F respectively (Munshi *et al.* 2013). This results in the muramyl-pentapeptide product, UDP-MurNAc/Glyc-L-Ala-D-IsoGlu-*m*-DAP-D-Ala-D-Ala, also known as Park's nucleotide (Kurosu *et al.* 2007). Despite the different amino acid specificities, the four ligases share common properties: the reaction mechanism; six invariant 'Mur' residues; an ATP-binding consensus; three-dimensional structural domains (Barreateau *et al.* 2008). Due to these similarities, it is plausible that a single inhibitor could target more than one Mur ligase and such inhibitors have been reported in the literature (Tomasic *et al.* 2010). Numerous small molecule inhibitors of the Mur



ligases have been discovered and are the subject of an extensive review (Hrast *et al.* 2014). In most cases, the inhibitors were identified from high-throughput screening (HTS) campaigns of compound libraries employing *in vitro* kinetic assays. These types of *in vitro* screening methods are limited in use against *Mtb* Mur ligases given that only MurC and MurE have been biochemically characterized (Mahapatra *et al.* 2000; Li *et al.* 2011). This dictates the next rational step towards the target-based discovery of Mur ligase inhibitors. Ddl is the target of D-cycloserine (Bruning *et al.* 2011), a second-line drug used in the treatment of TB, and is at the cornerstone of treatment for MDR and extensively drug resistant (XDR)-TB. D-cycloserine acts as a structural analogue of D-Ala, inhibiting the binding of either D-Ala to Ddl (Prosser and de Carvalho, 2013a, b).

The first membrane-anchored peptidoglycan precursor is generated by the translocation of Park's nucleotide to decaprenyl phosphate (C<sub>50</sub>-P), catalysed by MurX (also known as MraY), forming Lipid I (Kurosu *et al.* 2007). There are a number of nucleoside-based complex natural products that inhibit MurX, including muraymycin, liposidomycin, caprazamycin and capuramycin (Dini, 2005). Capuramycin and derivatives exhibit killing *in vitro* and *in vivo* and more significantly, analogues of capuramycin have been shown to kill non-replicating *Mtb*, a feature not common to the majority of cell wall biosynthesis inhibitors (Koga *et al.* 2004; Reddy *et al.* 2008; Nikonenko *et al.* 2009; Siricilla *et al.* 2015). Significantly, the analogue SQ641 is in preclinical development (<http://www.newtbdrugs.org>).

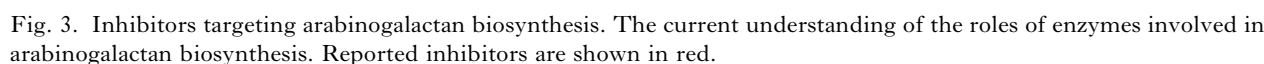
The final intracellular step of peptidoglycan synthesis is performed by the glycosyltransferase, MurG. A  $\beta(1 \rightarrow 4)$  linkage between GlcNAc (from UDP-GlcNAc) and MurNAc/Glyc of Lipid I is formed, leading to the generation of Lipid II, the monomeric building block of peptidoglycan (Mengin-Lecreulx *et al.* 1991). A library of transition state mimics have been designed for *Escherichia coli* MurG, and tested against *Mtb* MurG with partial success, one being the first inhibitor identified against the *Mtb* enzyme (Trunkfield *et al.* 2010).

The enzyme catalysing the translocation of Lipid II across the plasma membrane has been the subject of much debate. To date, there is evidence for two different enzymes with 'flippase' activity: MurJ and FtsW (Ruiz, 2008, 2015; Mohammadi *et al.* 2011, 2014; Sham *et al.* 2014). Further biochemical characterization is required to confirm the identification of the 'flippase'. Inhibitors against this enzyme would be expected to exhibit broad-spectrum activity, targeting a vital activity in all bacteria.

Following translocation across the plasma membrane, Lipid II is polymerized by the monofunctional and bifunctional Penicillin-binding proteins

(PBPs) (Sauvage *et al.* 2008). Bifunctional PBPs (PonA1/PBP1 and PonA2/PBP2) possess transglycosylase and transpeptidase domains. The former domain is responsible for linking the disaccharide building blocks of Lipid II to the pre-existing glycan chains (with the concomitant release of decaprenyl pyrophosphate), whereas the latter domain catalyses the formation of the classical (3  $\rightarrow$  4) cross-links, between *m*-DAP and D-Ala of the adjacent pentapeptide chains, with the cleavage of the terminal D-Ala. D,D-transpeptidation and D,D-carboxypeptidation is performed by the monofunctional PBPs, both resulting in the cleavage of the terminal D-Ala of the peptide stem (Goffin and Ghuysen, 2002). Only 20% of the cross-links in *Mtb* peptidoglycan are (3  $\rightarrow$  4) (Kumar *et al.* 2012). The majority are (3  $\rightarrow$  3) links between two tetrapeptide stems, with the release of the fourth position D-Ala (Lavollay *et al.* 2008). This reaction is catalysed by the L,D-transpeptidases, with D,D-carboxypeptidation as a prerequisite activity. The L,D-transpeptidases are structurally unrelated to PBPs, with different active site residues (cysteine and serine, respectively) (Mainardi *et al.* 2005; Biarrotte-Sorin *et al.* 2006). The  $\beta$ -lactam antibiotics have been used in the treatment of bacterial infections for nearly a century, and gave rise to the discovery of their target, the PBPs. The L,D-transpeptidases are resistant to most  $\beta$ -lactam antibiotics, except the carbapenems (Dubee *et al.* 2012). Until recently,  $\beta$ -lactams were not considered for use in the treatment of TB, due to the expression of a broad-spectrum  $\beta$ -lactamase, BlaC. However, it has been shown that BlaC is irreversibly inactivated by clavulanic acid, yet hydrolyses carbapenems at a low rate (Hugonnet *et al.* 2009). Combined treatment of the  $\beta$ -lactam with the  $\beta$ -lactamase inhibitor has been shown to be bactericidal against both replicating and non-replicating forms of *Mtb*, and combinations are now being explored in clinical trials (Hugonnet *et al.* 2009; Rullas *et al.* 2015). A well-documented inhibitor of the transglycosylase of PBPs, moenomycin (van Heijenoort *et al.* 1987), a natural product glycolipid, is yet to have proven efficacy against *Mtb*.

The inhibitors discussed thus far directly target the enzymes involved in peptidoglycan biosynthesis. There are, however, other antibiotics that act on the peptidoglycan precursors. For example, the glycopeptides, vancomycin and teicoplanin, bind to the D-Ala-D-Ala terminus of the pentapeptide stem, preventing polymerization reactions (Reynolds, 1989). Members of the lantibiotic family of antibiotics, such as nisin, interact with the pyrophosphate moiety of Lipid II, forming a pore in the cytoplasmic membrane, but also inhibiting peptidoglycan biosynthesis (Wiedemann *et al.* 2001). The lipoglycopeptide ramoplanin inhibits the action of MurG by binding to Lipid I. Ramoplanin also binds to Lipid II, preventing its polymerization (Lo *et al.* 2000).



The major cell wall polysaccharide, arabinogalactan (Fig. 1), as the name suggests, is composed of galactose and arabinose sugar residues, in the furanose (*f*) ring form (Gal*f*) (McNeil *et al.* 1987). Arabinogalactan is attached to peptidoglycan via a single linker unit (McNeil *et al.* 1990). The galactan component is a linear chain of approximately 30 alternating 5- and 6-linked  $\beta$ -D-Gal*f* residues (Daffe *et al.* 1990). Three highly branched arabinan chains, consisting of approximately 30 Araf residues, are attached to the galactan chain (Besra *et al.* 1995). The non-reducing termini of the arabinan chains act as an attachment site for mycolic acids, succinyl and galactosamine (D-GalN) moieties (Draper *et al.* 1997; Bhamidi *et al.* 2008).

Arabinogalactan biosynthesis is illustrated in Fig. 3. The first committed step begins in the cytoplasm and proceeds by the formation of the linker unit connecting peptidoglycan to arabinogalactan, which is initiated by WecA, a GlcNAc-1-P transferase (Jin *et al.* 2010). This enzyme catalyses the transfer of GlcNAc-1-P to C<sub>50</sub>-P. WbbL, a rhamnosyltransferase catalyses the transfer of L-rhamnose (L-Rha) from dTDP-L-Rha to position 3 of C<sub>50</sub>-P-P-GlcNAc to form C<sub>50</sub>-P-P-GlcNAc-L-Rha, completing the linker unit (McNeil *et al.* 1990; Mills *et al.* 2004). WecA has been identified as the target of caprazamycin derivatives, such as CPZEN-45, with the original nucleoside antibiotic shown to target Mray (Ishizaki *et al.* 2013). Recently, a fluorescence-based assay for WecA activity has been developed and used to screen compound libraries with some success (Mitachi *et al.* 2016). Inhibitors targeting WbbL have yet to be identified. This essential enzyme, present in all mycobacteria, is recognized as a promising target and efforts are

The linker unit provides an attachment point for the polymerization of the galactan chain. This process also occurs in the cytoplasm. The bifunctional galactofuranosyltransferases (GlfT1 and GlfT2) (Alderwick *et al.* 2008) are responsible for the synthesis of the linear galactan chain. Initially, GlfT1 transfers Galf from UDP-Galf to the C-4 position of L-Rha, and then adds a second Galf residue to the C-5 position of the primary Galf, generating C<sub>50</sub>-P-P-GlcNAc-L-Rha-Galf<sub>2</sub> (Mikusova *et al.* 2006; Alderwick *et al.* 2008; Belanova *et al.* 2008). GlfT2 sequentially transfers Galf residues to the growing galactan chain with alternating  $\beta$  (1  $\rightarrow$  5) and  $\beta$ (1  $\rightarrow$  6) glycosidic linkages (Kremer *et al.* 2001a; Rose *et al.* 2006). The galactan chains contain ~30 Galf residues *in vivo*, forming C<sub>50</sub>-P-P-GlcNAc-L-Rha-Galf<sub>30</sub> (Daffe *et al.* 1990), but the chain length determination mechanism is yet to be fully understood. GlfT1 and GlfT2 are suitable targets, as rationalized by an *in silico* target identification program (Raman *et al.* 2008). UDP-Galf derivatives, with modifications to the C-5 and C-6 positions have been investigated as suitable inhibitors of these enzymes, whereby they cause premature galactan chain termination (Peltier *et al.* 2010).

The remainder of arabinogalactan synthesis occurs on the outside of the cell. Although the transport mechanism of this cell wall polysaccharide is not fully understood, Rv3781 and Rv3783, encoding an ABC transporter, are potential ‘flippase’ candidates (Dianiskova *et al.* 2011). Araf residues are transferred directly onto C<sub>50</sub>-P-P-GlcNAc-L-Rha-Galf<sub>30</sub> from the lipid donor decaprenylphosphoryl-D-arabinose (DPA) (Wolucka *et al.* 1994). DPA is synthesized through a series of cytoplasmic steps, and originates exclusively from phospho- $\alpha$ -D-ribosyl-1-pyrophosphate (pRpp), prior to reorientation to the extracellular

face of the plasma membrane. The pRpp synthetase, PrsA, catalyses the transfer of pyrophosphate from ATP to C-1 of ribose-5-phosphate, forming pRpp (Alderwick *et al.* 2011b). A decaprenyl moiety is added, catalysed by UbiA (decaprenol-1-phosphate 5-phosphoribosyltransferase), forming decaprenol-1-monophosphate 5-phosphoribose (Alderwick *et al.* 2005; Huang *et al.* 2005, 2008). Rv3807c encodes a putative phospholipid phosphatase, which catalyses C-5 dephosphorylation, generating decaprenol-1-phosphoribose (DPR) (Jiang *et al.* 2011). Finally, DPA is generated by an epimerization reaction of the ribose C-2 hydroxyl, catalysed by a two-step oxidation/reduction activity of the decaprenylphosphoribose-2'-epimerase consisting of subunits DprE1 and DprE2 (Mikusova *et al.* 2005).

The DPA synthetic pathway is a validated drug target. The nitro-benzothiazinones (BTZs) and the structurally related dinitrobenzamides target DprE1 and are effective against MDR and XDR strains of *Mtb* with low toxicity (Christophe *et al.* 2009; Batt *et al.* 2012; Makarov *et al.* 2014, 2015). The success of these compounds has led to the study of the other enzymes as potential drug targets. Conditional knockdown mutants of *dprE1*, *dprE2*, *ubiA*, *prsA* and *Rv3807c* have proven the essentiality of all except *Rv3807c*, and a target-based whole-cell screen has been developed using these strains of reduced expression levels to identify enzyme-specific inhibitors. Inhibitors targeting a particular enzyme cause increased sensitivity and this was confirmed with BTZ and KRT2029 targeting DprE1 and UbiA, respectively, and can be the subject of future medicinal chemistry efforts (Kolly *et al.* 2014).

The mechanism of DPA reorientation into the periplasm is unknown. The 'flippase' was recently considered to be Rv3789, but there is evidence that this protein plays a different role: to act as an anchor protein to recruit AftA (Kolly *et al.* 2015). AftA is the first arabinofuranosyltransferase (AraT), of a predicted six, to commence the addition of arabinose from DPA onto the galactan chain (Alderwick *et al.* 2006). AftA transfers a single Araf residue onto C-5 of  $\beta(1 \rightarrow 6)$  Galf residues 8, 10 and 12 of C<sub>50</sub>-P-P-GlcNAc-L-Rha-Galf<sub>30</sub> (Alderwick *et al.* 2005). EmbA and EmbB, so called because their discovery was based on the mode of action elucidation of ethambutol (EMB), catalyse the addition of further  $\alpha(1 \rightarrow 5)$  Araf polymerization (Alderwick *et al.* 2005). AftC introduces  $\alpha(1 \rightarrow 3)$  branching (Birch *et al.* 2008), with AftD having an equivalent role (Skovierova *et al.* 2009). The structure terminates in a well-defined hexa-arabinofuranosyl (Araf<sub>6</sub>) structural motif: [ $\beta$ -D-Araf-(1  $\rightarrow$  2)- $\alpha$ -D-Araf]<sub>2</sub>-3,5- $\alpha$ -D-Araf-(1  $\rightarrow$  5)- $\alpha$ -D-Araf. This motif is generated by EmbA, EmbB, AftC, AftD and AftB (Escuyer *et al.* 2001; Alderwick *et al.* 2005; Birch *et al.* 2008, 2010; Skovierova *et al.* 2009). AftB

catalyses the transfer of the terminal  $\beta(1 \rightarrow 2)$  Araf residues (Seidel *et al.* 2007). C-5 of the terminal  $\beta$ -D-Araf and the penultimate 2- $\alpha$ -D-Araf of this motif act as anchoring points for mycolic acids (McNeil *et al.* 1991).

The Emb arabinosyltransferases are inhibited by EMB, a well-recognized anti-TB drug, which is employed in the short-course treatment strategy of TB. Efforts are focused on investigating EMB analogues, such as SQ109 (Jia *et al.* 2005a, b, c; Sacksteder *et al.* 2012) and SQ775 (Bogatcheva *et al.* 2006), for future lead drug development. Interestingly, the other AraTs are not inhibited by EMB (Alderwick *et al.* 2006; Seidel *et al.* 2007; Birch *et al.* 2008) and screening for inhibitors against these enzymes is hindered due to the nature of the protein and substrate (membrane bound). However, there have been reports on the development of DPA analogues for the inhibition of arabinogalactan biosynthesis (Pathak *et al.* 2001; Owen *et al.* 2007). A recent study employing a cell free assay approach with membrane preparations has determined that various DPA analogues are able to limit the incorporation of a radiolabelled DP[<sup>14</sup>C]A (Zhang *et al.* 2011).

The primary structure of arabinogalactan is completed by the transfer of succinyl and D-GalN residues to the inner arabinan units. PpgS, polyprenyl-phospho-N-acetylgalactosaminyl synthase, catalyses the formation of polyprenol-P-D-GalNAc from polyprenyl-P and UDP-GalNAc, which is then translocated across the membrane (Skovierova *et al.* 2010; Rana *et al.* 2012). The deacylation to polyprenol-P-D-GalN occurs in an undetermined location and by an unknown mechanism. The glycosyltransferase, Rv3779, transfers D-GalN to arabinogalactan at the C-2 position of 3,5-branched Araf residue (Scherman *et al.* 2009; Skovierova *et al.* 2010; Peng *et al.* 2012; Rana *et al.* 2012). Succinylated Araf residues have also been detected at this position of non-mycolated arabinan chains (Bhamidi *et al.* 2008), but the enzyme responsible is currently unknown. A comprehensive mechanistic and functional understanding of these enzymes is required for evaluation as suitable drug targets and to date, there are no identified inhibitors against these processes. The final stage is the attachment of the arabinogalactan macromolecule to peptidoglycan. The enzyme responsible for this essential ligation has recently been elucidated to be Lcp1 (Harrison *et al.* 2016).

#### PHOSPHATIDYL-MYO-INOSITOL MANNOSIDES, LIPOMANNAN AND LIPOARABINOMANNAN

The glycolipids, phosphatidyl-*myo*-inositol mannosides (PIMs), and the related lipoglycans, lipomannan (LM) and lipoarabinomannan (LAM), are non-covalently anchored into the inner and outer



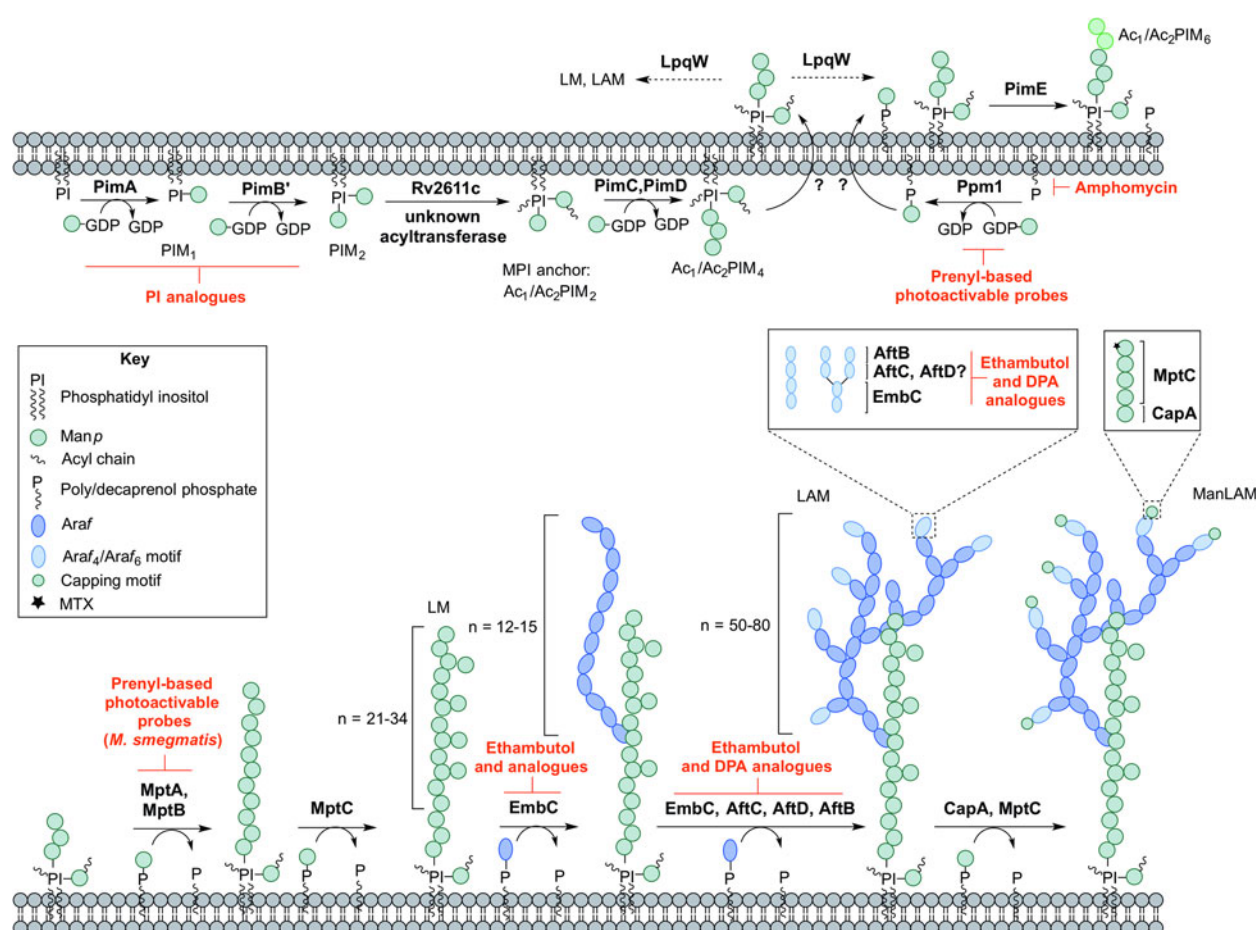


Fig. 4. Inhibitors targeting the biosynthesis of phosphatidyl-*myo*-inositol mannosides, lipomannan and lipoarabinomannan. The current understanding of the biosynthesis of PIMs, LM, LAM and ManLAM. Reported inhibitors are shown in red.

membranes of the cell wall *via* the phosphatidyl-*myo*-inositol unit (Ortalo-Magne *et al.* 1996) (Fig. 1). The core structure of PIM consists of an acylated *sn*-glycerol-3-phospho-(1-*D*-*myo*-inositol), the phosphatidyl inositol (PI) unit. Glycosylation with mannopyranose (Man<sub>p</sub>) residues at the O-2 and O-6 positions of *myo*-inositol, results in the mannosyl phosphate inositol (MPI) anchor (Ballou *et al.* 1963; Ballou and Lee, 1964; Nigou *et al.* 2004). The MPI structure is highly diverse, with variations in the type (commonly palmitic and tuberculostearic chains (Pitarque *et al.* 2005)), number and location of acyl chains. The most prevalent forms of PIMs in mycobacteria are tri- and tetra-acylated phospho-*myo*-inositol di/hexamannosides (Ac<sub>1</sub>PIM<sub>2</sub>, Ac<sub>1</sub>PIM<sub>6</sub>, Ac<sub>2</sub>PIM<sub>2</sub>, Ac<sub>2</sub>PIM<sub>6</sub>), where in the hexamannosides, there is one Man<sub>p</sub> unit on the O-2 and five Man<sub>p</sub> units on the O-6 position of *myo*-inositol (Gilleron *et al.* 2001). Extensions of mannan and arabinomannan chains on the MPI anchor form LM and LAM, respectively. In both LM and LAM, the mannan chain consists of approximately 21–34  $\alpha(1 \rightarrow 6)$  linked Man<sub>p</sub> units, decorated with single  $\alpha(1 \rightarrow 2)$ -Man<sub>p</sub> residues (Kaur *et al.* 2008). In LAM, the mannan chain is

glycosylated through an  $\alpha(1 \rightarrow 2)$  linkage with ~50–80 Araf residues (Khoo *et al.* 1996).

In mycobacteria, PI and PIMs contribute up to 56% of all phospholipids in the cell wall and 37% in the cytoplasmic membrane (Goren, 1984). These significant quantities indicate their importance. Not only are they structural components, they also have roles in cell wall integrity, permeability and control of septation and division (Parish *et al.* 1997; Patterson *et al.* 2003; Fukuda *et al.* 2013). LM and LAM are involved in *Mtb* pathogenicity, with evidence to suggest they are modulators of host-pathogen interactions (Schlesinger *et al.* 1994; Nigou *et al.* 2002; Maeda *et al.* 2003). These features of PIMs, LM and LAM make them suitable targets in anti-TB drug discovery.

#### BIOSYNTHESIS OF PHOSPHATIDYL-*MYO*-INOSITOL MANNOSIDES, LIPOMANNAN AND LIPARABINOMANNAN

PIM biosynthesis begins in the cytoplasm (Fig. 4). The  $\alpha$ -mannopyranosyl transferase (Man<sub>p</sub>T), PimA, of the GT-A/B superfamily, transfers Man<sub>p</sub> from the donor GDP-Man<sub>p</sub> to position O-2 of the



*myo*-inositol ring to form PIM<sub>1</sub> (Kordulakova *et al.* 2002; Guerin *et al.* 2007). A second Manp residue is transferred to position O-6 of the *myo*-inositol ring by PimB' to form PIM<sub>2</sub> (Guerin *et al.* 2009). Acylation of the Manp residue of PIM<sub>1</sub> is performed by the acyltransferase Rv2611c before or after the addition of the second Manp residue (Kordulakova *et al.* 2003). The acylation of the C-3 position of the *myo*-inositol ring is performed by an unknown acyltransferase. This finishes the synthesis of the MPI anchor. Mannosylation of Ac<sub>1</sub>/Ac<sub>2</sub>PIM<sub>2</sub> to Ac<sub>1</sub>/Ac<sub>2</sub>PIM<sub>3</sub> is performed by a ManpT, designated PimC, but this enzyme is yet to be confirmed in *Mtb* H37Rv (Kremer *et al.* 2002b). It is suspected that the subsequent addition of Manp to the non-reducing end of Ac<sub>1</sub>/Ac<sub>2</sub>PIM<sub>3</sub> is performed by the unidentified PimC or PimD forming Ac<sub>1</sub>/Ac<sub>2</sub>PIM<sub>4</sub>. The ManpTs have been the subject of target-based screening programs. More specifically, *in vitro* PimA activity was screened with approximately 350 compounds. Several hit molecules exhibited significant inhibition, but the compounds did not exhibit *in vivo* activity in *Mtb* (Sipos *et al.* 2015). Substrate analogues of PimA and PimB', galactose-derived phosphonate analogs of PI, have also been developed, which show enzyme inhibition in a cell-free system (Dinev *et al.* 2007).

The biosynthesis of Ac<sub>1</sub>/Ac<sub>2</sub>PIM<sub>4</sub> marks the transition towards the synthesis of higher order PIMs, LM and LAM (Fig. 4). It is predicted that the synthesis of Ac<sub>1</sub>/Ac<sub>2</sub>PIM<sub>4</sub> occurs on the cytoplasmic side of the membrane, and at this point, is flipped across the membrane by an unidentified translocase, with the remainder of the steps thought to occur in the periplasmic space. The integral membrane ManpTs (of the GT-C glycosyltransferase superfamily) are reliant on polyprenyl-phosphate-based mannose donors (PPM) rather than the nucleotide-based sugars (Berg *et al.* 2007). The polyprenol monophosphomannose synthase, Ppm1, catalyses the synthesis of PPM from GDP-Manp and polyprenol phosphates (Gurcha *et al.* 2002).

PimE catalyses the transfer of an  $\alpha(1 \rightarrow 2)$ -linked Manp residue onto Ac<sub>1</sub>/Ac<sub>2</sub>PIM<sub>4</sub>, generating Ac<sub>1</sub>/Ac<sub>2</sub>PIM<sub>5</sub> (Morita *et al.* 2006). The transfer of the last Manp residue is either performed by PimE or by an unidentified GT-C glycosyltransferase forming Ac<sub>1</sub>/Ac<sub>2</sub>PIM<sub>6</sub> (Morita *et al.* 2006). The distal 2-linked Manp residues are not present in the mannan core of LM or LAM; Ac<sub>1</sub>/Ac<sub>2</sub>PIM<sub>4</sub> is the likely precursor for the extension of the mannan chain. Recent evidence suggests that the putative lipoprotein LpqW channels intermediates such as Ac<sub>1</sub>/Ac<sub>2</sub>PIM<sub>4</sub> towards either PimE (to form the polar lipids) or to LM and LAM synthesis (Crellin *et al.* 2008). The mannosyltransferases, MptA and MptB (Mishra *et al.* 2007, 2008), are responsible for the  $\alpha(1 \rightarrow 6)$ -linked mannan core of LM and LAM. MptC catalyses the transfer of the

monomannose side chains via  $\alpha(1 \rightarrow 2)$  linkages, forming mature LM (Kaur *et al.* 2008; Mishra *et al.* 2011). Modification of LM leads to LAM. Approximately 50–80 Araf residues are added using DPA as the donor, comparable to that of the arabinogalactan domain. An unidentified ArafT primes the mannan chain, which is further elongated by EmbC, adding 12–16 Araf residues with  $\alpha(1 \rightarrow 5)$  linkages (Shi *et al.* 2006; Alderwick *et al.* 2011a). AftC, the same enzyme involved in arabinogalactan synthesis, integrates  $\alpha(1 \rightarrow 3)$  Araf branches (Birch *et al.* 2008). It has also been speculated that AftD introduces  $\alpha(1 \rightarrow 3)$  Araf, but its function is yet to be confirmed (Skovierova *et al.* 2009). The arabinan domain is terminated by  $\beta(1 \rightarrow 2)$  Araf linkages, predicted to be performed by AftB, resulting in branched hexa-arabino- or linear tetra-arabino- side motifs. Further structural heterogeneity is introduced by capping motifs. These moieties consist of a number of  $\alpha(1 \rightarrow 2)$ -linked Manp residues, producing mannosylated LAM (ManLAM) (Kaur *et al.* 2008). Using PPM, the  $\alpha(1 \rightarrow 5)$  ManpT, CapA, attaches the first Manp residue (Dinadayala *et al.* 2006). MptC catalyses the addition of subsequent  $\alpha(1 \rightarrow 2)$  Manp residues (Kaur *et al.* 2008), which can be decorated with an  $\alpha(1 \rightarrow 4)$ -linked 5-deoxy-5-methyl-thio-xylofuranose (MTX) residue (Ludwiczak *et al.* 2002; Turnbull *et al.* 2004). The enzymes involved in the addition of MTX and succinyl residues to LAM are still to be determined.

The essentiality of PPM in lipoglycan biosynthesis makes Ppm1 an attractive drug target. Amphomycin, a lipopeptide antibiotic, inhibits the synthesis of PPM by sequestering the polyprenol phosphates, and consequently inhibits the extracellular ManpTs (Banerjee *et al.* 1981; Besra *et al.* 1997). Guy *et al.* (2004) designed a variety of prenyl-based photoactivable probes. Upon photoactivation, a number of the probes exhibited inhibitory activity against *Mtb* Ppm1 and *M. smegmatis*  $\alpha(1 \rightarrow 6)$  ManpTs (Guy *et al.* 2004). Substrate analogues of the ManpTs have been designed to investigate enzyme–substrate interactions and mechanisms of action (Brown *et al.* 2001; Tam and Lowary, 2010). These types of studies will provide an invaluable insight into the interactions involved and for the future design of inhibitors.

#### MYCOLIC ACIDS

The final distinctive component of the mycobacterial cell wall is the unique fatty acids, termed the mycolic acids (Fig. 1). These unique long chain  $\alpha$ -alkyl- $\beta$ -hydroxy fatty acids (comprised a meromycolate chain of C<sub>42</sub>–C<sub>62</sub> and a long saturated  $\alpha$ -chain C<sub>24</sub>–C<sub>26</sub>) are attached to the arabinogalactan layer, but also make up other outer cell envelope lipids such as trehalose mono/di-mycolates and glucose

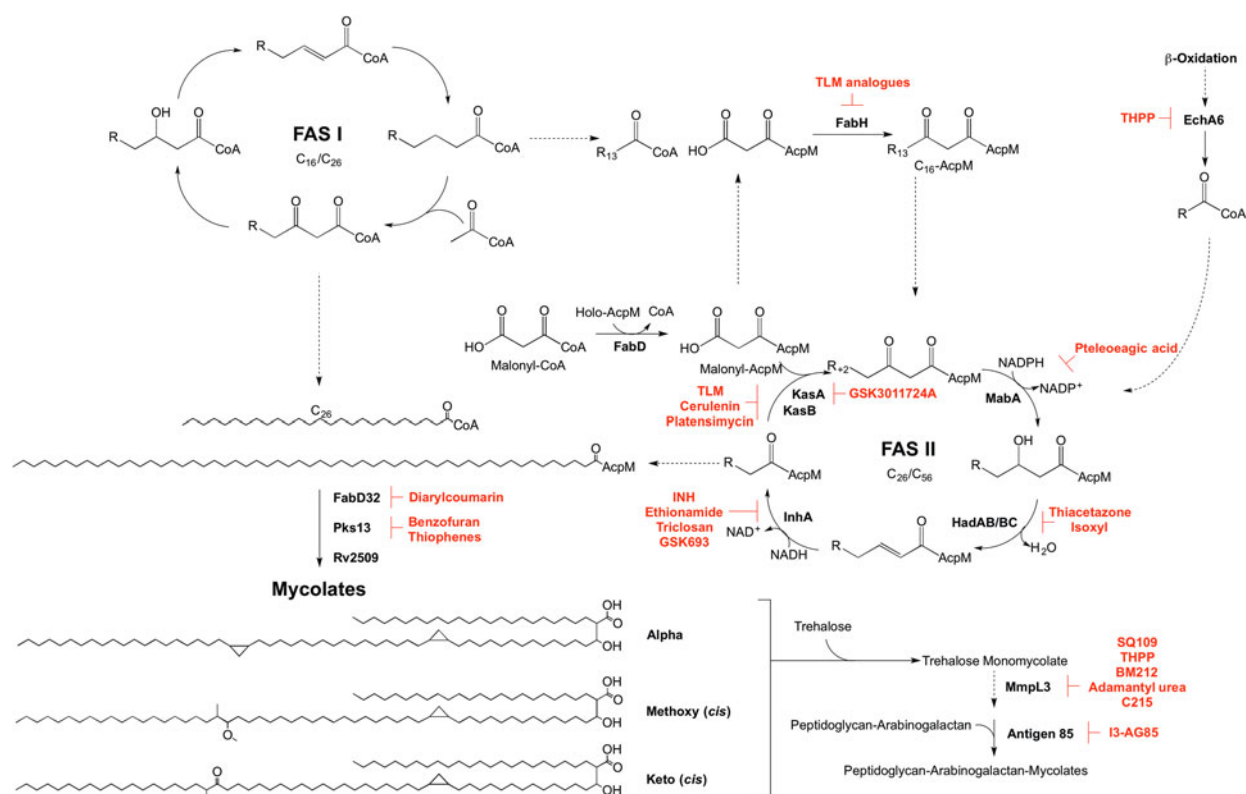


Fig. 5. Inhibitors targeting mycolic acid biosynthesis. The enzymes involved in the mycolic acid biosynthetic pathway are presented. Reported inhibitors are shown in red. 'R' represents an acyl chain of varying carbon units in length.

monomycolate. There are three subclasses of mycolic acids: α-mycolates, containing cyclopropane rings in the *cis*-configuration; methoxy-mycolates and keto-mycolates containing methoxy or ketone groups, respectively, and have cyclopropane rings in the *cis*- or *trans*- configuration (Brennan and Nikaido, 1995; Watanabe *et al.* 2001, 2002). Mycolic acids contribute to the permeability of the cell wall, and as such are essential for cell viability, and are also essential in virulence, making the biosynthesis of mycolates suitable drug targets (Liu *et al.* 1996).

#### MYCOLIC ACID BIOSYNTHESIS

Mycolic acid biosynthesis occurs in the cytoplasm, involving two distinct pathways, termed fatty acid synthase types I and II (FAS I and FAS II) (Fig. 5). FAS I (Rv2524c), a multifunctional polypeptide, generates short-chain fatty acyl-CoA esters that can either form the saturated α-branch (C<sub>24</sub>), or be extended by FAS II to form the meromycolate chain (Cole *et al.* 1998). Elongation of the fatty acids is dependent on the availability of holo-AcpM, an acyl carrier protein, and malonyl-CoA. FabD, the malonyl:AcpM transacylase generates malonyl-AcpM (Kremer *et al.* 2001b). C<sub>14</sub>-CoA primers from FAS I are condensed with malonyl-AcpM, catalysed by FabH (β-ketoacyl ACP

synthase) (Choi *et al.* 2000), forming a pivotal link between the FAS I and FAS II pathways. The C<sub>16</sub>-AcpM formed is channeled to the FAS II pathway (Bhatt *et al.* 2007), where it undergoes a round of keto-reduction, dehydration and enoyl-reduction, catalysed by: MabA, a β-ketoacyl-AcpM reductase (Marrakchi *et al.* 2002); HadAB/BC, a β-hydroxyacyl-AcpM hydratase (Sacco *et al.* 2007); InhA, an enoyl-AcpM reductase (Banerjee *et al.* 1994). Successive cycles ensue, whereby the condensation reaction of FabH is replaced by the activities of KasA and KasB, β-ketoacyl synthases (Schaeffer *et al.* 2001; Kremer *et al.* 2002a). The AcpM-bound acyl chain extends by two carbon units in each cycle, forming a saturated long-chain meromycolate of C<sub>42</sub>–C<sub>62</sub>, which is subject to modifications such as *cis*-/*trans*-cyclopropanation, and the addition of methoxy and keto groups (Dubnau *et al.* 2000; Glickman *et al.* 2000; Glickman, 2003; Barkan *et al.* 2010). FabD32, a fatty acyl-AMP ligase, activates the meromycolate chain (Trivedi *et al.* 2004) and the subsequent meromycolyl-AMP is linked with the α-alkyl-CoA ester, catalysed by Pks13, to generate a α-alkyl-β-keto-mycolic acid (Gande *et al.* 2004; Portevin *et al.* 2004). Finally a reduction step, catalysed by Rv2509, generates a mature mycolate (Bhatt *et al.* 2008). Transport of the mycolates to either the cell envelope or for attachment to arabinogalactan remains to be elucidated. It is considered that

the mycolates are transported in the form of trehalose monomycolate (TMM). In the generation of TMM, Takayama *et al.* (2005) propose that a mycolyl-transferase transfers the mycolyl group from mycolyl-Pks13 to D-mannopyranosyl-1-phosphoheptaprenol (Besra *et al.* 1994). The mycolyl group of mycolyl-D-mannopyranosyl-1-phosphoheptaprenol is transferred to trehalose-6-phosphate by a second mycolyltransferase, forming TMM-phosphate. The phosphate moiety is removed by a trehalose-6-phosphate phosphatase, and the TMM is immediately translocated outside of the cell using a resistance-nodulation-division (RND) family of efflux pumps, termed mycobacterial membrane proteins large (MmpL), limiting TMM accumulation in the cytoplasm (Takayama *et al.* 2005; Grzegorzewicz *et al.* 2012; Varela *et al.* 2012). Finally, the mycolyltransferase Antigen 85 complex, formed of Ag85A, Ag85B and Ag85C, attaches the mycolic acid moiety from TMM to arabinogalactan (Jackson *et al.* 1999). This complex also catalyses the formation of trehalose dimycolate, TDM, from two TMM molecules with the release of trehalose (Takayama *et al.* 2005). TDM, or 'cord factor', is implicated in the pathogenicity of *Mtb*.

The enzymes involved in mycolic acid biosynthesis are the targets of numerous inhibitors. In 1952, shortly after its discovery, isoniazid (INH) was administered as a front-line and essential antibiotic in the treatment of TB (Medical Research Council, 1952) and has only recently had the mode of action elucidated. Initially thought to target KatG due to mutations in the corresponding gene in resistant isolates (Zhang and Young, 1994; Rouse and Morris, 1995), INH was later revealed to be a pro-drug, with the true target being InhA (Banerjee *et al.* 1994; Larsen *et al.* 2002). Ethionamide, a structural analogue of INH, also requires cellular activation via EthA, before targeting InhA (Banerjee *et al.* 1994). Direct inhibitors of InhA that do not require activation are now being searched for (Lu *et al.* 2010; Vilcheze *et al.* 2011; Pan and Tonge, 2012; Encinas *et al.* 2014; Manjunatha *et al.* 2015; Sink *et al.* 2015; Martinez-Hoyos *et al.* 2016). One such molecule is the broad-spectrum antibiotic triclosan, which has not been adopted in TB treatment due to its sub-optimal bioavailability (Wang *et al.* 2004). In the last year, GlaxoSmithKline have published a set of thiadiazole compounds, which directly target InhA, with GSK693 demonstrating *in vivo* efficacy comparable to INH (Martinez-Hoyos *et al.* 2016). Therefore, old drug targets should not be discounted in the search for new anti-tubercular agents.

The  $\beta$ -ketoacyl synthases, KasA and KasB, are the targets of the natural products cerulenin (Parrish *et al.* 1999; Schaeffer *et al.* 2001; Kremer *et al.* 2002a), platensimycin (Brown *et al.* 2009), and thiolactomycin (TLM) (Kremer *et al.* 2000; Schaeffer *et al.* 2001).

There has been significant interest in TLM due to its broad-spectrum activity and numerous analogues have been synthesized to improve on potency and pharmacokinetic properties (Kremer *et al.* 2000; Senior *et al.* 2003, 2004; Kim *et al.* 2006). The biphenyl-based 5-substituents of TLM also exhibit *in vitro* activity against FabH, but with no whole-cell activity (Senior *et al.* 2003, 2004). The 2-tosyl-naphthalene-1,4-diol pharmacophore of TLM also has *in vitro* activity against FabH, however, whole-cell data are yet to be published (Alhamadsheh *et al.* 2008). Recently, a new anti-TB compound, an indazole sulfonamide GSK3011724A, was discovered from a phenotypic whole-cell HTS (Abrahams *et al.* 2016). The compound was shown to target KasA specifically, with no discernable target engagement with KasB or FabH, and is currently the focus of medicinal chemistry optimization (Abrahams *et al.* 2016).

Due to the success of InhA as a chemotherapeutic target, there is a mounting interest in the other enzymes involved in mycolic acid biosynthesis from a drug target perspective that could bypass INH resistance in MDR and XDR-TB. Formerly used in the treatment of TB, the thiocarbamide-containing drugs, thiacetazone and isoxyl, were shown to target mycolic acid biosynthesis and the inhibition mechanism has recently been elucidated. Following activation by EthA, both drugs target the HadA subunit of the HadABC dehydratase, forming a covalent interaction with the active site cysteine (Grzegorzewicz *et al.* 2015). It has also been shown that thiacetazone inhibits cyclopropanation of mycolic acids (Alahari *et al.* 2007). MabA has been the subject of a molecular docking study. Comparable with the control inhibitory substrate isonicotinic-acyl-NADH, pteleoellagic acid had a high docking score with *in vivo* activity to be confirmed (Shilpi *et al.* 2015). Through a target-based screening approach linked with whole-genome sequencing of resistant mutants, a benzofuran has been shown to target Pks13 (Ioerger *et al.* 2013). Additionally, Pks13 is the target of thiophene compounds (Wilson *et al.* 2013) including 2-aminothiophenes (Thanna *et al.* 2016). From a GFP reporter-based whole-cell HTS, a diarylcoumarin exhibited potent activity against *Mtb* and this structural class was shown to target FadD32 by inhibiting the acyl-acyl carrier protein synthetase activity (Stanley *et al.* 2013). The homologue of the Rv2509 reductase in *M. smegmatis* is non-essential but loss of function increases susceptibility to lipophilic antibiotics such as rifampicin. Targeting this 'secondary' drug target in *Mtb* could increase the susceptibility of the bacilli to antibiotics (Bhatt *et al.* 2008). The Antigen 85 complex has been the focus of a number of inhibitor-based screening studies (Belisle *et al.* 1997; Gobec *et al.* 2004; Sanki *et al.* 2008, 2009; Elamin *et al.* 2009; Barry *et al.* 2011). Recently, an inhibitor from a compound library was shown to bind to Antigen 85C, and derivatives of this compound

have been synthesized, with 2-amino-6-propyl-4,5,6,7-tetrahydro-1-benzothiphen-3-carbonitrile (I3-AG85) exhibiting the lowest MIC in *Mtb* and drug-resistant strains (Warrier *et al.* 2012).

In the target identification of new anti-tubercular compounds, some targets can be regarded as promiscuous, inhibited by multiple different chemical scaffolds, exemplified by MmpL3 (Grzegorzewicz *et al.* 2012; La Rosa *et al.* 2012; Stanley *et al.* 2012; Tahlan *et al.* 2012; Lun *et al.* 2013; Remuinan *et al.* 2013), a predicted TMM transporter. Through the generation and sequencing of spontaneous resistant mutants, a number of inhibitors with diverse chemical structures have been shown to target MmpL3 (Grzegorzewicz *et al.* 2012; La Rosa *et al.* 2012; Stanley *et al.* 2012; Tahlan *et al.* 2012; Lun *et al.* 2013; Remuinan *et al.* 2013). However, a recent chemoproteomics approach determined that one of the proposed inhibitor classes of MmpL3, the tetrahydropyrazo[1,5-a]pyrimidine-3-carboxamides (THPPs), has a novel alternative target, EchA6 (Cox *et al.* 2016). Sequence analysis predicted EchA6 to be an enoyl-CoA hydratase, but it lacks the residues required for catalytic activity. Through an extensive biochemical investigation, Cox *et al.* (2016) predicted that EchA6 shuttles fatty acyl-CoA esters from the  $\beta$ -oxidation pathway into FAS II, ready for the condensation activities of KasA or KasB with malonyl-AcpM. This research demonstrates that target identification of inhibitory compounds can unveil not only a new biological pathway, but also an untapped area for drug targets.

#### DRUG DISCOVERY EFFORTS

The strategies involved in drug discovery are forever evolving. Traditional enzyme screening campaigns and medicinal chemistry focused on ligand-based inhibitor designs (such as substrate or transition state analogues) that once dominated drug discovery are being superseded by phenotypic HTS. The former approach often relies on the X-ray crystal structure of the enzyme or biochemical understanding, and successful inhibitors from these screens are further challenged by target engagement *in vivo*. Over recent years, HTS has become the lead approach in drug discovery. HTS employs extensive compound libraries of diverse chemical structures, and as a consequence, these methods can identify a multitude of inhibitors with novel chemical scaffolds. Phenotypic HTS can reveal anti-TB agents with whole-cell activity and unknown modes of action, having the potential to unveil new biochemical pathways (Abrahams *et al.* 2012, 2016; Gurucha *et al.* 2014; Mugumbate *et al.* 2015). Alternatively, phenotypic HTS can be target-based, focusing on enzymes or pathways such as those involved in cell wall biosynthesis. This can

be a very effective way to identify novel anti-TB compounds with known modes of action, but is limited by the specified target (Batt *et al.* 2015; Martinez-Hoyos *et al.* 2016). Target assignment is a fundamental step in the drug discovery pipeline. Without knowledge of the physiological target, efforts can be wasted on developing compounds against an unsuitable target, such as those homologous in humans. Establishing the mode of action of an inhibitor is a prerequisite for facilitating medicinal chemistry efforts to convert compounds into potential drug candidates.

#### Concluding remarks

The essential mycobacterial cell wall, responsible for structural integrity, permeability and pathogenicity, is an attractive drug target, both structurally and biosynthetically. Recent advancements in biochemical and omics-based techniques have led to the discovery and mechanistic understanding of enzymes involved in mycobacterial cell wall synthesis and assembly. Although a number of key enzymes are yet to be established, there are a plethora of suitable targets, exploited not only in current treatment programmes but also for anti-TB drug discovery. In the current TB treatment regimen, two of the front-line drugs, INH and EMB, target mycolic acid and arabinogalactan biosynthesis, respectively, with the second-line drugs such as ethionamide and D-cycloserine also targeting cell wall production. The proven success of these drugs validates the future development of inhibitors targeting the unique mycobacterial cell wall, which remains a source of unexploited clinically relevant drug targets. The continued progression in drug discovery approaches and the optimization of biochemical techniques, will enable the rapid identification of anti-TB agents, many of which are likely to target the biosynthesis of the so-called ‘Achilles heel’ of *Mtb*.

#### ACKNOWLEDGEMENTS

The authors would like to thank Jonathan Cox for his technical support and advice.

#### FINANCIAL SUPPORT

G.S.B. acknowledges support in the form of a Personal Research Chair from Mr James Bardrick, a Royal Society Wolfson Research Merit Award, the Medical Research Council (MR/K012118/1) and the Wellcome Trust (081569/Z/06/Z).

#### REFERENCES

- Abrahams, K. A., Cox, J. A., Spivey, V. L., Loman, N. J., Pallen, M. J., Constantinidou, C., Fernandez, R., Alemparte, C., Remuinan, M. J., Barros, D., Balcells, L. and Besra, G. S. (2012). Identification of novel imidazo[1,2-a]pyridine inhibitors targeting *M. tuberculosis* QcrB. *PLoS ONE* 7, e52951.



- Abrahams, K. A., Chung, C. W., Ghidelli-Disse, S., Rullas, J., Rebollo-Lopez, M. J., Gurcha, S. S., Cox, J. A., Mendoza, A., Jimenez-Navarro, E., Martinez-Martinez, M. S., Neu, M., Shillings, A., Homes, P., Argyrou, A., Casanueva, R., Loman, N. J., Moynihan, P. J., Lelievre, J., Selenski, C., Axtman, M., Kremer, L., Bantscheff, M., Angulo-Barturen, I., Izquierdo, M. C., Cammack, N. C., Drewes, G., Ballell, L., Barros, D., Besra, G. S. and Bates, R. H. (2016). Identification of KasA as the cellular target of an anti-tubercular scaffold. *Nature Communications* **7**, 12581.
- Alahari, A., Trivelli, X., Guerardel, Y., Dover, L. G., Besra, G. S., Sacchettini, J. C., Reynolds, R. C., Coxon, G. D. and Kremer, L. (2007). Thiacetazone, an antitubercular drug that inhibits cyclopropanation of cell wall mycolic acids in mycobacteria. *PLoS ONE* **2**, e1343.
- Alderwick, L. J., Radmacher, E., Seidel, M., Gande, R., Hitchen, P. G., Morris, H. R., Dell, A., Sahm, H., Eggeling, L. and Besra, G. S. (2005). Deletion of Cg-emb in corynebacteriaceae leads to a novel truncated cell wall arabinogalactan, whereas inactivation of Cg-ubiA results in an arabinan-deficient mutant with a cell wall galactan core. *Journal of Biological Chemistry* **280**, 32362–32371.
- Alderwick, L. J., Seidel, M., Sahm, H., Besra, G. S. and Eggeling, L. (2006). Identification of a novel arabinofuranosyltransferase (AftA) involved in cell wall arabinan biosynthesis in *Mycobacterium tuberculosis*. *Journal of Biological Chemistry* **281**, 15653–15661.
- Alderwick, L. J., Dover, L. G., Veerapen, N., Gurcha, S. S., Kremer, L., Roper, D. L., Pathak, A. K., Reynolds, R. C. and Besra, G. S. (2008). Expression, purification and characterisation of soluble GlfT and the identification of a novel galactofuranosyltransferase Rv3782 involved in priming GlfT-mediated galactan polymerisation in *Mycobacterium tuberculosis*. *Protein Expression and Purification* **58**, 332–341.
- Alderwick, L. J., Lloyd, G. S., Ghadbane, H., May, J. W., Bhatt, A., Eggeling, L., Futterer, K. and Besra, G. S. (2011a). The C-terminal domain of the Arabinosyltransferase *Mycobacterium tuberculosis* EmbC is a lectin-like carbohydrate binding module. *PLoS Pathogens* **7**, e1001299.
- Alderwick, L. J., Lloyd, G. S., Lloyd, A. J., Lovering, A. L., Eggeling, L. and Besra, G. S. (2011b). Biochemical characterization of the *Mycobacterium tuberculosis* phosphoribosyl-1-pyrophosphate synthetase. *Glycobiology* **21**, 410–425.
- Alhmadshah, M. M., Waters, N. C., Sachdeva, S., Lee, P. and Reynolds, K. A. (2008). Synthesis and biological evaluation of novel sulfonyl-naphthalene-1,4-diols as FabH inhibitors. *Bioorganic and Medicinal Chemistry Letters* **18**, 6402–6405.
- Ballou, C. E. and Lee, Y. C. (1964). The structure of a myoinositol mannoside from *Mycobacterium tuberculosis* glycolipid. *Biochemistry* **3**, 682–685.
- Ballou, C. E., Vilkas, E. and Lederer, E. (1963). Structural studies on the myo-inositol phospholipids of *Mycobacterium tuberculosis* (var. bovis, strain BCG). *Journal of Biological Chemistry* **238**, 69–76.
- Banerjee, A., Dubnau, E., Quemard, A., Balasubramanian, V., Um, K. S., Wilson, T., Collins, D., de Lisle, G. and Jacobs, W. R., Jr. (1994). *inhA*, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*. *Science* **263**, 227–230.
- Banerjee, D. K., Scher, M. G. and Waechter, C. J. (1981). Amphomycin: effect of the lipopeptide antibiotic on the glycosylation and extraction of dolichyl monophosphate in calf brain membranes. *Biochemistry* **20**, 1561–1568.
- Barkan, D., Rao, V., Sukenick, G. D. and Glickman, M. S. (2010). Redundant function of *cmaA2* and *mmaA2* in *Mycobacterium tuberculosis* cis cyclopropanation of oxygenated mycolates. *Journal of Bacteriology* **192**, 3661–3668.
- Barreateau, H., Kovac, A., Boniface, A., Sova, M., Gobec, S. and Blanot, D. (2008). Cytoplasmic steps of peptidoglycan biosynthesis. *FEMS Microbiology Reviews* **32**, 168–207.
- Barry, C. S., Backus, K. M., Barry, C. E., III and Davis, B. G. (2011). ESI-MS assay of *M. tuberculosis* cell wall antigen 85 enzymes permits substrate profiling and design of a mechanism-based inhibitor. *Journal of the American Chemical Society* **133**, 13232–13235.
- Batt, S. M., Jabeen, T., Bhowruth, V., Quill, L., Lund, P. A., Eggeling, L., Alderwick, L. J., Futterer, K. and Besra, G. S. (2012). Structural basis of inhibition of *Mycobacterium tuberculosis* DprE1 by benzothiazinone inhibitors. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 11354–11359.
- Batt, S. M., Izquierdo, M. C., Pichel, J. C., Stubbs, C. J., Del Peral, L. V.-G., Perez-Herran, E., Dhar, N., Mouzon, B., Rees, M., Hutchinson, J. P., Young, R. J., McKinney, J. D., Barros-Aguirre, D., Ballell Pages, L., Besra, G. S. and Argyrou, A. (2015). Whole cell target engagement identifies novel inhibitors of *Mycobacterium tuberculosis* decaprenylphosphoryl- $\beta$ -D-ribose oxidase. *ACS Infectious Diseases* **1**, 615–626.
- Belanova, M., Dianiskova, P., Brennan, P. J., Completo, G. C., Rose, N. L., Lowary, T. L. and Mikusova, K. (2008). Galactosyl transferases in mycobacterial cell wall synthesis. *Journal of Bacteriology* **190**, 1141–1145.
- Belisle, J. T., Vissa, V. D., Sievert, T., Takayama, K., Brennan, P. J. and Besra, G. S. (1997). Role of the major antigen of *Mycobacterium tuberculosis* in cell wall biogenesis. *Science* **276**, 1420–1422.
- Berg, S., Kaur, D., Jackson, M. and Brennan, P. J. (2007). The glycosyltransferases of *Mycobacterium tuberculosis* – roles in the synthesis of arabinogalactan, lipoarabinomannan, and other glycoconjugates. *Glycobiology* **17**, 35–56R.
- Besra, G. S., Sievert, T., Lee, R. E., Slayden, R. A., Brennan, P. J. and Takayama, K. (1994). Identification of the apparent carrier in mycolic acid synthesis. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 12735–12739.
- Besra, G. S., Khoo, K. H., McNeil, M. R., Dell, A., Morris, H. R. and Brennan, P. J. (1995). A new interpretation of the structure of the mycolyl-arabinogalactan complex of *Mycobacterium tuberculosis* as revealed through characterization of oligoglycosylalditol fragments by fast-atom bombardment mass spectrometry and <sup>1</sup>H nuclear magnetic resonance spectroscopy. *Biochemistry* **34**, 4257–4266.
- Besra, G. S., Morehouse, C. B., Rittner, C. M., Waechter, C. J. and Brennan, P. J. (1997). Biosynthesis of mycobacterial lipoarabinomannan. *Journal of Biological Chemistry* **272**, 18460–18466.
- Bhamidi, S., Scherman, M. S., Rithner, C. D., Prenni, J. E., Chatterjee, D., Khoo, K. H. and McNeil, M. R. (2008). The identification and location of succinyl residues and the characterization of the interior arabinan region allow for a model of the complete primary structure of *Mycobacterium tuberculosis* mycolyl arabinogalactan. *Journal of Biological Chemistry* **283**, 12992–13000.
- Bhatt, A., Molle, V., Besra, G. S., Jacobs, W. R., Jr. and Kremer, L. (2007). The *Mycobacterium tuberculosis* FAS-II condensing enzymes: their role in mycolic acid biosynthesis, acid-fastness, pathogenesis and in future drug development. *Molecular Microbiology* **64**, 1442–1454.
- Bhatt, A., Brown, A. K., Singh, A., Minnikin, D. E. and Besra, G. S. (2008). Loss of a mycobacterial gene encoding a reductase leads to an altered cell wall containing beta-oxo-mycolic acid analogs and accumulation of ketones. *Chemistry and Biology* **15**, 930–939.
- Biarrotte-Sorin, S., Hugonnet, J. E., Delfosse, V., Mainardi, J. L., Gutmann, L., Arthur, M. and Mayer, C. (2006). Crystal structure of a novel beta-lactam-insensitive peptidoglycan transpeptidase. *Journal of Molecular Biology* **359**, 533–538.
- Birch, H. L., Alderwick, L. J., Bhatt, A., Rittmann, D., Krumbach, K., Singh, A., Bai, Y., Lowary, T. L., Eggeling, L. and Besra, G. S. (2008). Biosynthesis of mycobacterial arabinogalactan: identification of a novel alpha(1→3) arabinofuranosyltransferase. *Molecular Microbiology* **69**, 1191–1206.
- Birch, H. L., Alderwick, L. J., Appelmelk, B. J., Maaskant, J., Bhatt, A., Singh, A., Nigou, J., Eggeling, L., Geurtsen, J. and Besra, G. S. (2010). A truncated lipoglycan from mycobacteria with altered immunological properties. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 2634–2639.
- Bogatcheva, E., Hanrahan, C., Nikonenko, B., Samala, R., Chen, P., Gearhart, J., Barbosa, F., Einck, L., Nacy, C. A. and Protopopova, M. (2006). Identification of new diamine scaffolds with activity against *Mycobacterium tuberculosis*. *Journal of Medicinal Chemistry* **49**, 3045–3048.
- Brennan, P. J. and Nikaido, H. (1995). The envelope of mycobacteria. *Annual Review of Biochemistry* **64**, 29–63.
- Brown, A. K., Taylor, R. C., Bhatt, A., Futterer, K. and Besra, G. S. (2009). Platensimycin activity against mycobacterial beta-ketoacyl-ACP synthases. *PLoS ONE* **4**, e6306.
- Brown, J. R., Field, R. A., Barker, A., Guy, M., Grewal, R., Khoo, K. H., Brennan, P. J., Besra, G. S. and Chatterjee, D. (2001). Synthetic mannosides act as acceptors for mycobacterial alpha1-6 mannosyltransferase. *Bioorganic and Medicinal Chemistry* **9**, 815–824.
- Bruning, J. B., Murillo, A. C., Chacon, O., Barletta, R. G. and Sacchettini, J. C. (2011). Structure of the *Mycobacterium tuberculosis* D-alanine:D-alanine ligase, a target of the antituberculosis drug D-cycloserine. *Antimicrobial Agents and Chemotherapy* **55**, 291–301.
- Choi, K. H., Kremer, L., Besra, G. S. and Rock, C. O. (2000). Identification and substrate specificity of beta-ketoacyl (acyl carrier protein) synthase III (mtFabH) from *Mycobacterium tuberculosis*. *Journal of Biological Chemistry* **275**, 28201–28207.
- Christophe, T., Jackson, M., Jeon, H. K., Fenistein, D., Contreras-Dominguez, M., Kim, J., Genovesio, A., Carralot, J. P., Ewann, F., Kim, E. H., Lee, S. Y., Kang, S., Seo, M. J., Park, E. J., Skovierova, H., Pham, H., Riccardi, G., Nam, J. Y., Marsollier, L., Kempf, M., Joly-Guillou, M. L., Oh, T., Shin, W. K., No, Z.,

- Nehrbass, U., Brosch, R., Cole, S.T. and Brodin, P. (2009). High content screening identifies decaprenyl-phosphoribose 2' epimerase as a target for intracellular antimycobacterial inhibitors. *PLoS Pathogens* 5, e1000645.
- Cole, S.T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S.V., Eiglmeier, K., Gas, S., Barry, C.E., III, Tekalia, F., Badcock, K., Basham, D., Brown, D., Chillingworth, T., Connor, R., Davies, R., Devlin, K., Feltwell, T., Gentles, S., Hamlin, N., Holroyd, S., Hornsby, T., Jagels, K., Krogh, A., McLean, J., Moule, S., Murphy, L., Oliver, K., Osborne, J. et al. (1998). Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* 393, 537–544.
- Cox, J.A.G., Abrahams, K.A., Alemparte, C., Ghidelli-Disse, S., Rullas, J., Angulo-Barturen, I., Singh, A., Gurcha, S.S., Nataraj, V., Bethell, S., Remuñán, M.J., Encinas, L., Jervis, P.J., Cammack, N.C., Bhatt, A., Kruse, U., Bantscheff, M., Futterer, K., Barros, D., Ballell, L., Drewes, G. and Besra, G.S. (2016). THPP target assignment reveals EchA6 as an essential fatty acid shuttle in mycobacteria. *Nature Microbiology* 1, 1–10.
- Crellin, P.K., Kovacevic, S., Martin, K.L., Brammananth, R., Morita, Y.S., Billman-Jacobe, H., McConville, M.J. and Coppel, R.L. (2008). Mutations in *pimE* restore lipoarabinomannan synthesis and growth in a *Mycobacterium smegmatis* *lpqW* mutant. *Journal of Bacteriology* 190, 3690–3699.
- Daffe, M., Brennan, P.J. and McNeil, M. (1990). Predominant structural features of the cell wall arabinogalactan of *Mycobacterium tuberculosis* as revealed through characterization of oligoglycosyl alditol fragments by gas chromatography/mass spectrometry and by <sup>1</sup>H and <sup>13</sup>C NMR analyses. *Journal of Biological Chemistry* 265, 6734–6743.
- Dianiskova, P., Kordulakova, J., Skovierova, H., Kaur, D., Jackson, M., Brennan, P.J. and Mikusova, K. (2011). Investigation of ABC transporter from mycobacterial arabinogalactan biosynthetic cluster. *General Physiology and Biophysics* 30, 239–250.
- Dinadayala, P., Kaur, D., Berg, S., Amin, A.G., Vissa, V.D., Chatterjee, D., Brennan, P.J. and Crick, D.C. (2006). Genetic basis for the synthesis of the immunomodulatory mannose caps of lipoarabinomannan in *Mycobacterium tuberculosis*. *Journal of Biological Chemistry* 281, 20027–20035.
- Dinev, Z., Gannon, C.T., Egan, C., Watt, J.A., McConville, M.J. and Williams, S.J. (2007). Galactose-derived phosphonate analogues as potential inhibitors of phosphatidylinositol biosynthesis in mycobacteria. *Organic & Biomolecular Chemistry* 5, 952–959.
- Dini, C. (2005). MraY Inhibitors as novel antibacterial agents. *Current Topics in Medicinal Chemistry* 5, 1221–1236.
- Draper, P., Khoo, K.H., Chatterjee, D., Dell, A. and Morris, H.R. (1997). Galactosamine in walls of slow-growing mycobacteria. *Biochemical Journal* 327 (Pt 2), 519–525.
- Dubee, V., Triboulet, S., Mainardi, J.L., Etheve-Quelejeu, M., Gutmann, L., Marie, A., Dubost, L., Hugonnet, J.E. and Arthur, M. (2012). Inactivation of *Mycobacterium tuberculosis* 1,d-transpeptidase LdtMt(1) by carbapenems and cephalosporins. *Antimicrobial Agents and Chemotherapy* 56, 4189–4195.
- Dubnau, E., Chan, J., Raynaud, C., Mohan, V.P., Laneelle, M.A., Yu, K., Quemard, A., Smith, I. and Daffe, M. (2000). Oxygenated mycolic acids are necessary for virulence of *Mycobacterium tuberculosis* in mice. *Molecular Microbiology* 36, 630–637.
- Elamin, A.A., Stehr, M., Oehlmann, W. and Singh, M. (2009). The mycolyltransferase 85A, a putative drug target of *Mycobacterium tuberculosis*: development of a novel assay and quantification of glycolipid-status of the mycobacterial cell wall. *Journal of Microbiological Methods* 79, 358–363.
- Encinas, L., O'Keefe, H., Neu, M., Remuñan, M.J., Patel, A.M., Guardia, A., Davie, C.P., Perez-Macias, N., Yang, H., Convery, M.A., Messer, J.A., Perez-Herran, E., Centrella, P.A., Alvarez-Gomez, D., Clark, M.A., Huss, S., O'Donovan, G.K., Ortega-Muro, F., McDowell, W., Castaneda, P., Arico-Muendel, C.C., Pajk, S., Rullas, J., Angulo-Barturen, I., Alvarez-Ruiz, E., Mendoza-Losana, A., Ballell Pages, L., Castro-Pichel, J. and Evindar, G. (2014). Encoded library technology as a source of hits for the discovery and lead optimization of a potent and selective class of bactericidal direct inhibitors of *Mycobacterium tuberculosis* InhA. *Journal of Medicinal Chemistry* 57, 1276–1288.
- Escuyer, V.E., Lety, M.A., Torrelles, J.B., Khoo, K.H., Tang, J.B., Rithner, C.D., Frehel, C., McNeil, M.R., Brennan, P.J. and Chatterjee, D. (2001). The role of the *embA* and *embB* gene products in the biosynthesis of the terminal hexaarabinofuranosyl motif of *Mycobacterium smegmatis* arabinogalactan. *Journal of Biological Chemistry* 276, 48854–48862.
- Fukuda, T., Matsumura, T., Ato, M., Hamasaki, M., Nishiuchi, Y., Murakami, Y., Maeda, Y., Yoshimori, T., Matsumoto, S., Kobayashi, K., Kinoshita, T. and Morita, Y.S. (2013). Critical roles for lipomannan and lipoarabinomannan in cell wall integrity of mycobacteria and pathogenesis of tuberculosis. *American Society for Microbiology* 4, e00472–00412.
- Gande, R., Gibson, K.J., Brown, A.K., Krumbach, K., Dover, L.G., Sahm, H., Shioyama, S., Oikawa, T., Besra, G.S. and Eggeling, L. (2004). Acyl-CoA carboxylases (*accD2* and *accD3*), together with a unique polyketide synthase (*Cg-pks*), are key to mycolic acid biosynthesis in *Corynebacteriaceae* such as *Corynebacterium glutamicum* and *Mycobacterium tuberculosis*. *Journal of Biological Chemistry* 279, 44847–44857.
- Gillieron, M., Ronet, C., Mempel, M., Monsarrat, B., Gachelin, G. and Puzo, G. (2001). Acylation state of the phosphatidylinositol mannosides from *Mycobacterium bovis* bacillus Calmette Guérin and ability to induce granuloma and recruit natural killer T cells. *Journal of Biological Chemistry* 276, 34896–34904.
- Glickman, M.S. (2003). The *mmaA2* gene of *Mycobacterium tuberculosis* encodes the distal cyclopropane synthase of the alpha-mycolic acid. *Journal of Biological Chemistry* 278, 7844–7849.
- Glickman, M.S., Cox, J.S. and Jacobs, W.R., Jr. (2000). A novel mycolic acid cyclopropane synthetase is required for cording, persistence, and virulence of *Mycobacterium tuberculosis*. *Molecular Cell* 5, 717–727.
- Gobec, S., Plantan, I., Mravljak, J., Wilson, R.A., Besra, G.S. and Kikelj, D. (2004). Phosphonate inhibitors of antigen 85C, a crucial enzyme involved in the biosynthesis of the *Mycobacterium tuberculosis* cell wall. *Bioorganic and Medicinal Chemistry Letters* 14, 3559–3562.
- Goffin, C. and Ghuyssen, J.M. (2002). Biochemistry and comparative genomics of SxxK superfamily acyltransferases offer a clue to the mycobacterial paradox: presence of penicillin-susceptible target proteins versus lack of efficiency of penicillin as therapeutic agent. *Microbiology and Molecular Biology Reviews* 66, 702–738 (table of contents).
- Goren, M.B. (1984). Biosynthesis and structures of phospholipids and sulphatides. In *The Mycobacteria. A Sourcebook* (ed. Kubica, G.P. and Wayne, L.G.), pp. 379–415. Marcel Dekker Inc., New York.
- Grzegorzewicz, A.E., Ma, Y., Jones, V., Crick, D., Liav, A. and McNeil, M.R. (2008). Development of a microtitre plate-based assay for lipid-linked glycosyltransferase products using the mycobacterial cell wall rhamnosyltransferase WbbL. *Microbiology* 154, 3724–3730.
- Grzegorzewicz, A.E., Pham, H., Gundi, V.A., Scherman, M.S., North, E.J., Hess, T., Jones, V., Gruppo, V., Born, S.E., Kordulakova, J., Chavadi, S.S., Morisseau, C., Lenaerts, A.J., Lee, R.E., McNeil, M.R. and Jackson, M. (2012). Inhibition of mycolic acid transport across the *Mycobacterium tuberculosis* plasma membrane. *Nature Chemical Biology* 8, 334–341.
- Grzegorzewicz, A.E., Eynard, N., Quemard, A., North, E.J., Margolis, A., Lindenberger, J.J., Jones, V., Kordulakova, J., Brennan, P.J., Lee, R.E., Ronning, D.R., McNeil, M.R. and Jackson, M. (2015). Covalent modification of the *Mycobacterium tuberculosis* FAS-II dehydratase by Isoxyl and Thiacezone. *ACS Infectious Diseases* 1, 91–97.
- Guerin, M.E., Kordulakova, J., Schaeffer, F., Svetlikova, Z., Buschiazzi, A., Giganti, D., Gicquel, B., Mikusova, K., Jackson, M. and Alzari, P.M. (2007). Molecular recognition and interfacial catalysis by the essential phosphatidylinositol mannosyltransferase PimA from mycobacteria. *Journal of Biological Chemistry* 282, 20705–20714.
- Guerin, M.E., Kaur, D., Somashekar, B.S., Gibbs, S., Gest, P., Chatterjee, D., Brennan, P.J. and Jackson, M. (2009). New insights into the early steps of phosphatidylinositol mannoside biosynthesis in mycobacteria: PimB' is an essential enzyme of *Mycobacterium smegmatis*. *Journal of Biological Chemistry* 284, 25687–25696.
- Gurcha, S.S., Baulard, A.R., Kremer, L., Loch, C., Moody, D.B., Muhlecker, W., Costello, C.E., Crick, D.C., Brennan, P.J. and Besra, G.S. (2002). Ppm1, a novel polyprenol monophosphomannose synthase from *Mycobacterium tuberculosis*. *Biochemical Journal* 365, 441–450.
- Gurcha, S.S., Usha, V., Cox, J.A., Futterer, K., Abrahams, K.A., Bhatt, A., Alderwick, L.J., Reynolds, R.C., Loman, N.J., Nataraj, V., Alemparte, C., Barros, D., Lloyd, A.J., Ballell, L., Hobbath, J.V. and Besra, G.S. (2014). Biochemical and structural characterization of mycobacterial aspartyl-tRNA synthetase AspS, a promising TB drug target. *PLoS ONE* 9, e113568.
- Guy, M.R., Illarionov, P.A., Gurcha, S.S., Dover, L.G., Gibson, K.J., Smith, P.W., Minnikin, D.E. and Besra, G.S. (2004). Novel prenyl-linked benzophenone substrate analogues of mycobacterial mannosyltransferases. *Biochemical Journal* 382, 905–912.
- Harrison, J., Lloyd, G., Joe, M., Lowary, T.L., Reynolds, E., Walters-Morgan, H., Bhatt, A., Lovering, A., Besra, G.S. and Alderwick, L.J.

- (2016). Lcp1 is a phosphotransferase responsible for ligating arabinogalactan to peptidoglycan in *Mycobacterium tuberculosis*. *American Society for Microbiology* 7, e00972.
- Hingley-Wilson, S. M., Sambandamurthy, V. K. and Jacobs, W. R., Jr. (2003). Survival perspectives from the world's most successful pathogen, *Mycobacterium tuberculosis*. *Nature Immunology* 4, 949–955.
- Hrast, M., Sosic, I., Sink, R. and Gobec, S. (2014). Inhibitors of the peptidoglycan biosynthesis enzymes MurA–F. *Bioorganic Chemistry* 55, 2–15.
- Huang, H., Scherman, M. S., D'Haese, W., Vereecke, D., Holsters, M., Crick, D. C. and McNeil, M. R. (2005). Identification and active expression of the *Mycobacterium tuberculosis* gene encoding 5-phospho- $\alpha$ -D-ribose-1-diphosphate: decaprenyl-phosphate 5-phosphoribosyltransferase, the first enzyme committed to decaprenylphosphoryl-d-arabinose synthesis. *Journal of Biological Chemistry* 280, 24539–24543.
- Huang, H., Berg, S., Spencer, J. S., Vereecke, D., D'Haese, W., Holsters, M. and McNeil, M. R. (2008). Identification of amino acids and domains required for catalytic activity of DPPR synthase, a cell wall biosynthetic enzyme of *Mycobacterium tuberculosis*. *Microbiology* 154, 736–743.
- Hugonnet, J. E., Tremblay, L. W., Boshoff, H. I., Barry, C. E., III and Blanchard, J. S. (2009). Meropenem-clavulanate is effective against extensively drug-resistant *Mycobacterium tuberculosis*. *Science* 323, 1215–1218.
- Ioerger, T. R., O'Malley, T., Liao, R., Guinn, K. M., Hickey, M. J., Mohaideen, N., Murphy, K. C., Boshoff, H. I., Mizrahi, V., Rubin, E. J., Sassetti, C. M., Barry, C. E., III, Sherman, D. R., Parish, T. and Sacchettini, J. C. (2013). Identification of new drug targets and resistance mechanisms in *Mycobacterium tuberculosis*. *PLoS ONE* 8, e75245.
- Ishizaki, Y., Hayashi, C., Inoue, K., Igarashi, M., Takahashi, Y., Pujari, V., Crick, D. C., Brennan, P. J. and Nomoto, A. (2013). Inhibition of the first step in synthesis of the mycobacterial cell wall core, catalyzed by the GlcNAc-1-phosphate transferase WecA, by the novel caprazamycin derivative CPZEN-45. *Journal of Biological Chemistry* 288, 30309–30319.
- Jackson, M., Raynaud, C., Laneelle, M. A., Guilhot, C., Laurent-Winter, C., Ensergueix, D., Gicquel, B. and Daffe, M. (1999). Inactivation of the antigen 85C gene profoundly affects the mycolate content and alters the permeability of the *Mycobacterium tuberculosis* cell envelope. *Molecular Microbiology* 31, 1573–1587.
- Jia, L., Coward, L., Gorman, G. S., Noker, P. E. and Tomaszewski, J. E. (2005a). Pharmacoproteomic effects of isoniazid, ethambutol, and N-geranyl-N'-(2-Adamantyl)ethane-1,2-diamine (SQ109) on *Mycobacterium tuberculosis* H37Rv. *Journal of Pharmacology and Experimental Therapeutics* 315, 905–911.
- Jia, L., Tomaszewski, J. E., Hanrahan, C., Coward, L., Noker, P., Gorman, G., Nikonenko, B. and Protopopova, M. (2005b). Pharmacodynamics and pharmacokinetics of SQ109, a new diamine-based antitubercular drug. *British Journal of Pharmacology* 144, 80–87.
- Jia, L., Tomaszewski, J. E., Noker, P. E., Gorman, G. S., Glaze, E. and Protopopova, M. (2005c). Simultaneous estimation of pharmacokinetic properties in mice of three anti-tubercular ethambutol analogs obtained from combinatorial lead optimization. *Journal of Pharmaceutical and Biomedical Analysis* 37, 793–799.
- Jiang, T., He, L., Zhan, Y., Zang, S., Ma, Y., Zhao, X., Zhang, C. and Xin, Y. (2011). The effect of MSMEG\_6402 gene disruption on the cell wall structure of *Mycobacterium smegmatis*. *Microbial Pathogenesis* 51, 156–160.
- Jin, Y., Xin, Y., Zhang, W. and Ma, Y. (2010). *Mycobacterium tuberculosis* Rv1302 and *Mycobacterium smegmatis* MSMEG\_4947 have WecA function and MSMEG\_4947 is required for the growth of *M. smegmatis*. *FEMS Microbiology Letters* 310, 54–61.
- Kaur, D., Obregon-Henao, A., Pham, H., Chatterjee, D., Brennan, P. J. and Jackson, M. (2008). Lipoarabinomannan of *Mycobacterium*: mannose capping by a multifunctional terminal mannosyltransferase. *Proceedings of the National Academy of Sciences of the United States of America* 105, 17973–17977.
- Khoo, K. H., Douglas, E., Azadi, P., Inamine, J. M., Besra, G. S., Mikusova, K., Brennan, P. J. and Chatterjee, D. (1996). Truncated structural variants of lipoarabinomannan in ethambutol drug-resistant strains of *Mycobacterium smegmatis*. Inhibition of arabinan biosynthesis by ethambutol. *Journal of Biological Chemistry* 271, 28682–28690.
- Kim, D. H., Lees, W. J., Kempell, K. E., Lane, W. S., Duncan, K. and Walsh, C. T. (1996). Characterization of a Cys115 to Asp substitution in the *Escherichia coli* cell wall biosynthetic enzyme UDP-GlcNAc enolpyruvyl transferase (MurA) that confers resistance to inactivation by the antibiotic fosfomycin. *Biochemistry* 35, 4923–4928.
- Kim, P., Zhang, Y. M., Shenoy, G., Nguyen, Q. A., Boshoff, H. I., Manjunatha, U. H., Goodwin, M. B., Lonsdale, J., Price, A. C., Miller, D. J., Duncan, K., White, S. W., Rock, C. O., Barry, C. E., III and Dowd, C. S. (2006). Structure-activity relationships at the 5-position of thiolactomycin: an intact (5R)-isoprene unit is required for activity against the condensing enzymes from *Mycobacterium tuberculosis* and *Escherichia coli*. *Journal of Medicinal Chemistry* 49, 159–171.
- Koga, T., Fukuoka, T., Doi, N., Harasaki, T., Inoue, H., Hotoda, H., Kakuta, M., Muramatsu, Y., Yamamura, N., Hoshi, M. and Hirota, T. (2004). Activity of capuramycin analogues against *Mycobacterium tuberculosis*, *Mycobacterium avium* and *Mycobacterium intracellulare* in vitro and in vivo. *Journal of Antimicrobial Chemotherapy* 54, 755–760.
- Kolly, G. S., Boldrin, F., Sala, C., Dhar, N., Hartkoorn, R. C., Ventura, M., Serafini, A., McKinney, J. D., Manganelli, R. and Cole, S. T. (2014). Assessing the essentiality of the decaprenyl-phospho-d-arabinofuranose pathway in *Mycobacterium tuberculosis* using conditional mutants. *Molecular Microbiology* 92, 194–211.
- Kolly, G. S., Mukherjee, R., Kilascova, E., Abriata, L. A., Raccaud, M., Blasko, J., Sala, C., Dal Peraro, M., Mikusova, K. and Cole, S. T. (2015). GtrA protein Rv3789 is required for arabinosylation of arabinogalactan in *Mycobacterium tuberculosis*. *Journal of Bacteriology* 197, 3686–3697.
- Kordulakova, J., Gilleron, M., Mikusova, K., Puzo, G., Brennan, P. J., Gicquel, B. and Jackson, M. (2002). Definition of the first mannosylation step in phosphatidylinositol mannoside synthesis. PimA is essential for growth of mycobacteria. *Journal of Biological Chemistry* 277, 31335–31344.
- Kordulakova, J., Gilleron, M., Puzo, G., Brennan, P. J., Gicquel, B., Mikusova, K. and Jackson, M. (2003). Identification of the required acyltransferase step in the biosynthesis of the phosphatidylinositol mannosides of mycobacterium species. *Journal of Biological Chemistry* 278, 36285–36295.
- Kremer, L., Douglas, J. D., Baulard, A. R., Morehouse, C., Guy, M. R., Alland, D., Dover, L. G., Lakey, J. H., Jacobs, W. R., Jr., Brennan, P. J., Minnikin, D. E. and Besra, G. S. (2000). Thiolactomycin and related analogues as novel anti-mycobacterial agents targeting KasA and KasB condensing enzymes in *Mycobacterium tuberculosis*. *Journal of Biological Chemistry* 275, 16857–16864.
- Kremer, L., Dover, L. G., Morehouse, C., Hitchin, P., Everett, M., Morris, H. R., Dell, A., Brennan, P. J., McNeil, M. R., Flaherty, C., Duncan, K. and Besra, G. S. (2001a). Galactan biosynthesis in *Mycobacterium tuberculosis*. Identification of a bifunctional UDP-galactofuranosyltransferase. *Journal of Biological Chemistry* 276, 26430–26440.
- Kremer, L., Nampoothiri, K. M., Lesjean, S., Dover, L. G., Graham, S., Betts, J., Brennan, P. J., Minnikin, D. E., Loch, C. and Besra, G. S. (2001b). Biochemical characterization of acyl carrier protein (AcpM) and malonyl-CoA:AcpM transacylase (mtFabD), two major components of *Mycobacterium tuberculosis* fatty acid synthase II. *Journal of Biological Chemistry* 276, 27967–27974.
- Kremer, L., Dover, L. G., Carrere, S., Nampoothiri, K. M., Lesjean, S., Brown, A. K., Brennan, P. J., Minnikin, D. E., Loch, C. and Besra, G. S. (2002a). Mycolic acid biosynthesis and enzymic characterization of the beta-ketoacyl-ACP synthase A-condensing enzyme from *Mycobacterium tuberculosis*. *Biochemical Journal* 364, 423–430.
- Kremer, L., Gurcha, S. S., Bifani, P., Hitchin, P. G., Baulard, A., Morris, H. R., Dell, A., Brennan, P. J. and Besra, G. S. (2002b). Characterization of a putative alpha-mannosyltransferase involved in phosphatidylinositol trimannoside biosynthesis in *Mycobacterium tuberculosis*. *Biochemical Journal* 363, 437–447.
- Kumar, P., Arora, K., Lloyd, J. R., Lee, I. Y., Nair, V., Fischer, E., Boshoff, H. I. and Barry, C. E., III (2012). Meropenem inhibits D,D-carboxypeptidase activity in *Mycobacterium tuberculosis*. *Molecular Microbiology* 86, 367–381.
- Kumar, V., Saravanan, P., Arvind, A. and Mohan, C. G. (2011). Identification of hotspot regions of MurB oxidoreductase enzyme using homology modeling, molecular dynamics and molecular docking techniques. *Journal of Molecular Modeling* 17, 939–953.
- Kurosu, M., Mahapatra, S., Narayanasamy, P. and Crick, D. C. (2007). Chemoenzymatic synthesis of Park's nucleotide: toward the development of high-throughput screening for MraY inhibitors. *Tetrahedron Letters* 48, 799–803.
- La Rosa, V., Poce, G., Canseco, J. O., Buroni, S., Pasca, M. R., Biava, M., Raju, R. M., Porretta, G. C., Alfonso, S., Battilocchio, C., Javid, B., Sorrentino, F., Ioerger, T. R., Sacchettini, J. C., Manetti, F., Botta, M., De Logu, A., Rubin, E. J. and De Rossi, E. (2012). MmpL3 is the cellular target of the antitubercular pyrrole derivative BM212. *Antimicrobial Agents and Chemotherapy* 56, 324–331.
- Larsen, M. H., Vilcheze, C., Kremer, L., Besra, G. S., Parsons, L., Salfinger, M., Heifets, L., Hazbon, M. H., Alland, D., Sacchettini, J. C. and Jacobs, W. R., Jr. (2002). Overexpression of inhA, but not kasA, confers resistance to isoniazid and ethionamide in



- Mycobacterium smegmatis*, *M. bovis* BCG and *M. tuberculosis*. *Molecular Microbiology* **46**, 453–466.
- Lavollay, M., Arthur, M., Fourgeaud, M., Dubost, L., Marie, A., Veziris, N., Blanot, D., Gutmann, L. and Mainardi, J.L. (2008). The peptidoglycan of stationary-phase *Mycobacterium tuberculosis* predominantly contains cross-links generated by L,D-transpeptidation. *Journal of Bacteriology* **190**, 4360–4366.
- Li, Y., Zhou, Y., Ma, Y. and Li, X. (2011). Design and synthesis of novel cell wall inhibitors of *Mycobacterium tuberculosis* GlmM and GlmU. *Carbohydrate Research* **346**, 1714–1720.
- Liu, J., Barry, C.E., III, Besra, G.S. and Nikaido, H. (1996). Mycolic acid structure determines the fluidity of the mycobacterial cell wall. *Journal of Biological Chemistry* **271**, 29545–29551.
- Lo, M.C., Men, H., Branstrom, A., Helm, J., Yao, N., Goldman, R. and Walker, S. (2000). A new mechanism of action proposed for ramoplanin. *Journal of the American Chemical Society* **122**, 3540–3541.
- Lu, X.Y., You, Q.D. and Chen, Y.D. (2010). Recent progress in the identification and development of InhA direct inhibitors of *Mycobacterium tuberculosis*. *Mini Reviews in Medicinal Chemistry* **10**, 181–192.
- Ludwiczak, P., Gilleron, M., Bordat, Y., Martin, C., Gicquel, B. and Puzo, G. (2002). *Mycobacterium tuberculosis* phoP mutant: lipoarabinomannan molecular structure. *Microbiology* **148**, 3029–3037.
- Lun, S., Guo, H., Onajole, O.K., Pieroni, M., Gunosewoyo, H., Chen, G., Tipparaju, S.K., Ammerman, N.C., Kozikowski, A.P. and Bishai, W.R. (2013). Indoleamides are active against drug-resistant *Mycobacterium tuberculosis*. *Nature Communications* **4**, 2907.
- Maeda, N., Nigou, J., Herrmann, J.L., Jackson, M., Amara, A., Lagrange, P.H., Puzo, G., Gicquel, B. and Neyrolles, O. (2003). The cell surface receptor DC-SIGN discriminates between *Mycobacterium* species through selective recognition of the mannose caps on lipoarabinomannan. *Journal of Biological Chemistry* **278**, 5513–5516.
- Mahapatra, S., Crick, D.C. and Brennan, P.J. (2000). Comparison of the UDP-N-acetylmuramate:L-alanine ligase enzymes from *Mycobacterium tuberculosis* and *Mycobacterium leprae*. *Journal of Bacteriology* **182**, 6827–6830.
- Mahapatra, S., Scherman, H., Brennan, P.J. and Crick, D.C. (2005a). N Glycolylation of the nucleotide precursors of peptidoglycan biosynthesis of *Mycobacterium* spp. is altered by drug treatment. *Journal of Bacteriology* **187**, 2341–2347.
- Mahapatra, S., Yagi, T., Belisle, J.T., Espinosa, B.J., Hill, P.J., McNeil, M.R., Brennan, P.J. and Crick, D.C. (2005b). Mycobacterial lipid II is composed of a complex mixture of modified muramyl and peptide moieties linked to decaprenyl phosphate. *Journal of Bacteriology* **187**, 2747–2757.
- Mainardi, J.L., Fourgeaud, M., Hugonnet, J.E., Dubost, L., Brouard, J.P., Ouazzani, J., Rice, L.B., Gutmann, L. and Arthur, M. (2005). A novel peptidoglycan cross-linking enzyme for a beta-lactam-resistant transpeptidation pathway. *Journal of Biological Chemistry* **280**, 38146–38152.
- Makarov, V., Lechartier, B., Zhang, M., Neres, J., van der Sar, A.M., Raadsen, S.A., Hartkoorn, R.C., Ryabova, O.B., Vocat, A., Decosterd, L.A., Widmer, N., Buclin, T., Bitter, W., Andries, K., Pojer, F., Dyson, P.J. and Cole, S.T. (2014). Towards a new combination therapy for tuberculosis with next generation benzothiazinones. *EMBO Molecular Medicine* **6**, 372–383.
- Makarov, V., Neres, J., Hartkoorn, R.C., Ryabova, O.B., Kazakova, E., Sarkan, M., Huszar, S., Piton, J., Kolly, G.S., Vocat, A., Conroy, T.M., Mikusova, K. and Cole, S.T. (2015). The 8-pyrrole-benzothiazinones Are Noncovalent Inhibitors of DprE1 from *Mycobacterium tuberculosis*. *Antimicrobial Agents and Chemotherapy* **59**, 4446–4452.
- Manjunatha, U.H., Rao, S.P.S., Kondreddi, R.R., Noble, C.G., Camacho, L.R., Tan, B.H., Ng, S.H., Ng, P.S., Ma, N.L., Lakshminarayana, S.B., Herve, M., Barnes, S.W., Yu, W., Kuhen, K., Blasco, F., Beer, D., Walker, J.R., Tonge, P.J., Glynn, R., Smith, P.W. and Diagana, T.T. (2015). Direct inhibitors of InhA are active against *Mycobacterium tuberculosis*. *Science Translational Medicine* **7**, 269ra3.
- Marrakchi, H., Ducasse, S., Labesse, G., Montrozier, H., Margeat, E., Emorine, L., Charpentier, X., Daffe, M. and Quemard, A. (2002). MabA (FabG1), a *Mycobacterium tuberculosis* protein involved in the long-chain fatty acid elongation system FAS-II. *Microbiology* **148**, 951–960.
- Martinez-Hoyos, M., Perez-Herran, E., Gulten, G., Encinas, L., Alvarez-Gomez, D., Alvarez, E., Ferrer-Bazaga, S., Garcia-Perez, A., Ortega, F., Angulo-Barturen, I., Rullas-Trincado, J., Blanco Ruano, D., Torres, P., Castaneda, P., Huss, S., Fernandez Menendez, R., Gonzalez Del Valle, S., Ballell, L., Barros, D., Modha, S., Dhar, N., Signorino-Gelo, F., McKinney, J.D., Garcia-Bustos, J.F., Lavandera, J.L., Sacchetti, J.C., Jimenez, M.S., Martin-Casabona, N., Castro-Pichel, J. and Mendoza-Losana, A. (2016). Antitubercular drugs for an old target: GSK693 as a promising InhA direct inhibitor. *EBioMedicine* **8**, 291–301.
- McNeil, M., Wallner, S.J., Hunter, S.W. and Brennan, P.J. (1987). Demonstration that the galactosyl and arabinosyl residues in the cell-wall arabinogalactan of *Mycobacterium leprae* and *Mycobacterium tuberculosis* are furanoid. *Carbohydrate Research* **166**, 299–308.
- McNeil, M., Daffe, M. and Brennan, P.J. (1990). Evidence for the nature of the link between the arabinogalactan and peptidoglycan of mycobacterial cell walls. *Journal of Biological Chemistry* **265**, 18200–18206.
- McNeil, M., Daffe, M. and Brennan, P.J. (1991). Location of the mycolyl ester substituents in the cell walls of mycobacteria. *Journal of Biological Chemistry* **266**, 13217–13223.
- Medical Research Council (1952). Treatment of pulmonary tuberculosis with isoniazid; an interim report to the Medical Research Council by their Tuberculosis Chemotherapy Trials Committee. *British Medical Journal* **2**, 735–746.
- Mengin-Lecreux, D., Texier, L., Rousseau, M. and van Heijenoort, J. (1991). The murG gene of *Escherichia coli* codes for the UDP-N-acetylglucosamine: N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase involved in the membrane steps of peptidoglycan synthesis. *Journal of Bacteriology* **173**, 4625–4636.
- Mikusova, K., Huang, H., Yagi, T., Holsters, M., Vereecke, D., D'Haese, W., Scherman, M.S., Brennan, P.J., McNeil, M.R. and Crick, D.C. (2005). Decaprenylphosphoryl arabinofuranose, the donor of the D-arabinofuranosyl residues of mycobacterial arabinan, is formed via a two-step epimerization of decaprenylphosphoryl ribose. *Journal of Bacteriology* **187**, 8020–8025.
- Mikusova, K., Belanova, M., Kordulakova, J., Honda, K., McNeil, M.R., Mahapatra, S., Crick, D.C. and Brennan, P.J. (2006). Identification of a novel galactosyl transferase involved in biosynthesis of the mycobacterial cell wall. *Journal of Bacteriology* **188**, 6592–6598.
- Mills, J.A., Motichka, K., Jucker, M., Wu, H.P., Uhlik, B.C., Stern, R.J., Scherman, M.S., Vissa, V.D., Pan, F., Kundu, M., Ma, Y.F. and McNeil, M. (2004). Inactivation of the mycobacterial rhamnosyltransferase, which is needed for the formation of the arabinogalactan-peptidoglycan linker, leads to irreversible loss of viability. *Journal of Biological Chemistry* **279**, 43540–43546.
- Mio, T., Yabe, T., Arisawa, M. and Yamada-Okabe, H. (1998). The eukaryotic UDP-N-acetylglucosamine pyrophosphorylases. Gene cloning, protein expression, and catalytic mechanism. *Journal of Biological Chemistry* **273**, 14392–14397.
- Mishra, A.K., Alderwick, L.J., Rittmann, D., Tatituri, R.V., Nigou, J., Gilleron, M., Eggeling, L. and Besra, G.S. (2007). Identification of an alpha(1→6) mannopyranosyltransferase (MptA), involved in *Corynebacterium glutamicum* lipomannan biosynthesis, and identification of its orthologue in *Mycobacterium tuberculosis*. *Molecular Microbiology* **65**, 1503–1517.
- Mishra, A.K., Alderwick, L.J., Rittmann, D., Wang, C., Bhatt, A., Jacobs, W.R., Jr., Takayama, K., Eggeling, L. and Besra, G.S. (2008). Identification of a novel alpha(1→6) mannopyranosyltransferase MptB from *Corynebacterium glutamicum* by deletion of a conserved gene, NCgl1505, affords a lipomannan- and lipoarabinomannan-deficient mutant. *Molecular Microbiology* **68**, 1595–1613.
- Mishra, A.K., Krumbach, K., Rittmann, D., Appelmeik, B., Pathak, V., Pathak, A.K., Nigou, J., Geurtsen, J., Eggeling, L. and Besra, G.S. (2011). Lipoarabinomannan biosynthesis in *Corynebacterineae*: the interplay of two alpha(1→2)-mannopyranosyltransferases MptC and MptD in mannan branching. *Molecular Microbiology* **80**, 1241–1259.
- Mitachi, K., Siricilla, S., Yang, D., Kong, Y., Skorupinska-Tudek, K., Swiezewska, E., Franzblau, S.G. and Kurosu, M. (2016). Fluorescence-based assay for polyphosphoryl phosphate-GlcNAc-1-phosphate transferase (WecA) and identification of novel antimycobacterial WecA inhibitors. *Analytical Biochemistry* **512**, 78–90.
- Mohammadi, T., van Dam, V., Sijbrandi, R., Vernet, T., Zapun, A., Bouhss, A., Diepeveen-de Bruin, M., Nguyen-Disteche, M., de Kruijff, B. and Breukink, E. (2011). Identification of FtsW as a transporter of lipid-linked cell wall precursors across the membrane. *EMBO Journal* **30**, 1425–1432.
- Mohammadi, T., Sijbrandi, R., Lutters, M., Verheul, J., Martin, N.I., den Blaauwen, T., de Kruijff, B. and Breukink, E. (2014). Specificity of the transport of lipid II by FtsW in *Escherichia coli*. *Journal of Biological Chemistry* **289**, 14707–14718.



- Morita, Y. S., Sena, C. B., Waller, R. F., Kurokawa, K., Sernee, M. F., Nakatani, F., Haites, R. E., Billman-Jacobe, H., McConville, M. J., Maeda, Y. and Kinoshita, T. (2006). PimE is a polyprenol-phosphate-mannose-dependent mannosyltransferase that transfers the fifth mannose of phosphatidylinositol mannoside in mycobacteria. *Journal of Biological Chemistry* **281**, 25143–25155.
- Mugumbate, G., Abrahams, K. A., Cox, J. A., Papadatos, G., van Westen, G., Lelievre, J., Calus, S. T., Loman, N. J., Ballell, L., Barros, D., Overington, J. P. and Besra, G. S. (2015). Mycobacterial dihydrofolate reductase inhibitors identified using chemogenomic methods and *in vitro* validation. *PLoS ONE* **10**, e0121492.
- Munshi, T., Gupta, A., Evangelopoulos, D., Guzman, J. D., Gibbons, S., Keep, N. H. and Bhakta, S. (2013). Characterisation of ATP-dependent Mur ligases involved in the biogenesis of cell wall peptidoglycan in *Mycobacterium tuberculosis*. *PLoS ONE* **8**, e60143.
- Nigou, J., Gilleron, M., Rojas, M., Garcia, L. F., Thurnher, M. and Puzo, G. (2002). Mycobacterial lipoarabinomannans: modulators of dendritic cell function and the apoptotic response. *Microbes and Infection* **4**, 945–953.
- Nigou, J., Gilleron, M., Brando, T. and Puzo, G. (2004). Structural analysis of mycobacterial lipoglycans. *Applied Biochemistry and Biotechnology* **118**, 253–267.
- Nikonenko, B. V., Reddy, V. M., Protopopova, M., Bogatcheva, E., Einck, L. and Nacy, C. A. (2009). Activity of SQ641, a capuramycin analog, in a murine model of tuberculosis. *Antimicrobial Agents and Chemotherapy* **53**, 3138–3139.
- Ortalo-Magne, A., Lemassu, A., Laneelle, M. A., Bardou, F., Silve, G., Gounon, P., Marchal, G. and Daffe, M. (1996). Identification of the surface-exposed lipids on the cell envelopes of *Mycobacterium tuberculosis* and other mycobacterial species. *Journal of Bacteriology* **178**, 456–461.
- Owen, D. J., Davis, C. B., Hartnell, R. D., Madge, P. D., Thomson, R. J., Chong, A. K., Coppel, R. L. and von Itzstein, M. (2007). Synthesis and evaluation of galactofuranosyl N,N-dialkyl sulfenamides and sulfonamides as antimycobacterial agents. *Bioorganic and Medicinal Chemistry Letters* **17**, 2274–2277.
- Pan, P. and Tonge, P. J. (2012). Targeting InhA, the FASII enoyl-ACP reductase: SAR studies on novel inhibitor scaffolds. *Current Topics in Medicinal Chemistry* **12**, 672–693.
- Parish, T., Liu, J., Nikaido, H. and Stoker, N. G. (1997). A *Mycobacterium smegmatis* mutant with a defective inositol monophosphate phosphatase gene homolog has altered cell envelope permeability. *Journal of Bacteriology* **179**, 7827–7833.
- Parrish, N. M., Kuhajda, F. P., Heine, H. S., Bishai, W. R. and Dick, J. D. (1999). Antimycobacterial activity of cerulenin and its effects on lipid biosynthesis. *Journal of Antimicrobial Chemotherapy* **43**, 219–226.
- Pathak, A. K., Pathak, V., Khare, N. K., Maddry, J. A. and Reynolds, R. C. (2001). Synthesis of disaccharides related to the mycobacterial arabinogalactan. *Carbohydrate Letters* **4**, 117–122.
- Patterson, J. H., Waller, R. F., Jeevarajah, D., Billman-Jacobe, H. and McConville, M. J. (2003). Mannose metabolism is required for mycobacterial growth. *Biochemical Journal* **372**, 77–86.
- Peltier, P., Belanova, M., Dianiskova, P., Zhou, R., Zheng, R. B., Pearcey, J. A., Joe, M., Brennan, P. J., Nugier-Chauvin, C., Ferrieres, V., Lowary, T. L., Daniellou, R. and Mikusova, K. (2010). Synthetic UDP-furanoses as potent inhibitors of mycobacterial galactan biogenesis. *Chemistry and Biology* **17**, 1356–1366.
- Peneff, C., Ferrari, P., Charrier, V., Taburet, Y., Monnier, C., Zamboni, V., Winter, J., Harnois, M., Fassy, F. and Bourne, Y. (2001). Crystal structures of two human pyrophosphorylase isoforms in complexes with UDPGlc(Gal)NAc: role of the alternatively spliced insert in the enzyme oligomeric assembly and active site architecture. *EMBO Journal* **20**, 6191–6202.
- Peng, W., Zou, L., Bhamidi, S., McNeil, M. R. and Lowary, T. L. (2012). The galactosamine residue in mycobacterial arabinogalactan is alpha-linked. *Journal of Organic Chemistry* **77**, 9826–9832.
- Pitarque, S., Herrmann, J. L., Duteyrat, J. L., Jackson, M., Stewart, G. R., Lecointe, F., Payre, B., Schwartz, O., Young, D. B., Marchal, G., Lagrange, P. H., Puzo, G., Gicquel, B., Nigou, J. and Neyrolles, O. (2005). Deciphering the molecular bases of *Mycobacterium tuberculosis* binding to the lectin DC-SIGN reveals an underestimated complexity. *Biochemical Journal* **392**, 615–624.
- Portevin, D., De Sousa-D'Auria, C., Houssin, C., Grimaldi, C., Chami, M., Daffe, M. and Guilhot, C. (2004). A polyketide synthase catalyzes the last condensation step of mycolic acid biosynthesis in mycobacteria and related organisms. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 314–319.
- Prosser, G. A. and de Carvalho, L. P. (2013a). Kinetic mechanism and inhibition of *Mycobacterium tuberculosis* D-alanine:D-alanine ligase by the antibiotic D-cycloserine. *FEBS Journal* **280**, 1150–1166.
- Prosser, G. A. and de Carvalho, L. P. (2013b). Metabolomics reveal d-alanine:d-alanine ligase as the target of d-cycloserine in *Mycobacterium tuberculosis*. *ACS Medicinal Chemistry Letters* **4**, 1233–1237.
- Raman, K., Yeturu, K. and Chandra, N. (2008). TargetTB: a target identification pipeline for *Mycobacterium tuberculosis* through an interactome, reactome and genome-scale structural analysis. *BMC Systems Biology* **2**, 109.
- Rana, A. K., Singh, A., Gurcha, S. S., Cox, L. R., Bhatt, A. and Besra, G. S. (2012). Ppm1-encoded polyprenyl monophosphomannose synthase activity is essential for lipoglycan synthesis and survival in mycobacteria. *PLoS ONE* **7**, e48211.
- Rani, C. and Khan, I. A. (2016). UDP-GlcNAc pathway: potential target for inhibitor discovery against *M. tuberculosis*. *European Journal of Pharmaceutical Sciences* **83**, 62–70.
- Raymond, J. B., Mahapatra, S., Crick, D. C. and Pavelka, M. S., Jr. (2005). Identification of the namH gene, encoding the hydroxylase responsible for the N-glycolylation of the mycobacterial peptidoglycan. *Journal of Biological Chemistry* **280**, 326–333.
- Reddy, V. M., Einck, L. and Nacy, C. A. (2008). *In vitro* antimycobacterial activities of capuramycin analogues. *Antimicrobial Agents and Chemotherapy* **52**, 719–721.
- Remuinan, M. J., Perez-Herran, E., Rullas, J., Alemparte, C., Martinez-Hoyos, M., Dow, D. J., Afari, J., Mehta, N., Esquivias, J., Jimenez, E., Ortega-Muro, F., Fraile-Gabaldon, M. T., Spivey, V. L., Loman, N. J., Pallen, M. J., Constantinidou, C., Minick, D. J., Cacho, M., Rebollo-Lopez, M. J., Gonzalez, C., Sousa, V., Angulo-Barturen, I., Mendoza-Losana, A., Barros, D., Besra, G. S., Ballell, L. and Cammack, N. (2013). Tetrahydropyrazolo[1,5-a]pyrimidine-3-carboxamide and N-benzyl-6',7'-dihydrospiro[piperidine-4,4'-thieno[3,2-c]pyran] analogues with bactericidal efficacy against *Mycobacterium tuberculosis* targeting MmpL3. *PLoS ONE* **8**, e60933.
- Reynolds, P. E. (1989). Structure, biochemistry and mechanism of action of glycopeptide antibiotics. *European Journal of Clinical Microbiology and Infectious Diseases* **8**, 943–950.
- Rose, N. L., Completo, G. C., Lin, S. J., McNeil, M., Palcic, M. M. and Lowary, T. L. (2006). Expression, purification, and characterization of a galactofuranosyltransferase involved in *Mycobacterium tuberculosis* arabinogalactan biosynthesis. *Journal of the American Chemical Society* **128**, 6721–6729.
- Rouse, D. A. and Morris, S. L. (1995). Molecular mechanisms of isoniazid resistance in *Mycobacterium tuberculosis* and *Mycobacterium bovis*. *Infection and Immunity* **63**, 1427–1433.
- Ruiz, N. (2008). Bioinformatics identification of MurJ (MviN) as the peptidoglycan lipid II flippase in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 15553–15557.
- Ruiz, N. (2015). Lipid flippases for bacterial peptidoglycan biosynthesis. *Lipid Insights* **8**, 21–31.
- Rullas, J., Dhar, N., McKinney, J. D., Garcia-Perez, A., Lelievre, J., Diacon, A. H., Hugonnet, J. E., Arthur, M., Angulo-Barturen, I., Barros-Aguirre, D. and Ballell, L. (2015). Combinations of beta-lactam antibiotics currently in clinical trials are efficacious in a DHP-1-deficient mouse model of tuberculosis infection. *Antimicrobial Agents and Chemotherapy* **59**, 4997–4999.
- Sacco, E., Covarrubias, A. S., O'Hare, H. M., Carroll, P., Eynard, N., Jones, T. A., Parish, T., Daffe, M., Backbro, K. and Quemard, A. (2007). The missing piece of the type II fatty acid synthase system from *Mycobacterium tuberculosis*. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 14628–14633.
- Sacksteder, K. A., Protopopova, M., Barry, C. E., III, Andries, K. and Nacy, C. A. (2012). Discovery and development of SQ109: a new antitubercular drug with a novel mechanism of action. *Future Microbiology* **7**, 823–837.
- Sanki, A. K., Boucau, J., Srivastava, P., Adams, S. S., Ronning, D. R. and Sucheck, S. J. (2008). Synthesis of methyl 5-S-alkyl-5-thio-D-arabinofuranosides and evaluation of their antimycobacterial activity. *Bioorganic and Medicinal Chemistry* **16**, 5672–5682.
- Sanki, A. K., Boucau, J., Ronning, D. R. and Sucheck, S. J. (2009). Antigen 85C-mediated acyl-transfer between synthetic acyl donors and fragments of the arabinan. *Glycoconjugate Journal* **26**, 589–596.
- Sauvage, E., Kerff, F., Terrak, M., Ayala, J. A. and Charlier, P. (2008). The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. *FEMS Microbiology Reviews* **32**, 234–258.
- Schaeffer, M. L., Agnihotri, G., Volker, C., Kallender, H., Brennan, P. J. and Lonsdale, J. T. (2001). Purification and biochemical characterization of the *Mycobacterium tuberculosis* beta-ketoacyl-acyl

- carrier protein synthases KasA and KasB. *Journal of Biological Chemistry* **276**, 47029–47037.
- Scherman, H., Kaur, D., Pham, H., Skovierova, H., Jackson, M. and Brennan, P. J. (2009). Identification of a polyprenylphosphomannosyl synthase involved in the synthesis of mycobacterial mannositides. *Journal of Bacteriology* **191**, 6769–6772.
- Schlesinger, L. S., Hull, S. R. and Kaufman, T. M. (1994). Binding of the terminal mannosyl units of lipoarabinomannan from a virulent strain of *Mycobacterium tuberculosis* to human macrophages. *Journal of Immunology* **152**, 4070–4079.
- Seidel, M., Alderwick, L. J., Birch, H. L., Sahm, H., Eggeling, L. and Besra, G. S. (2007). Identification of a novel arabinofuranosyltransferase AftB involved in a terminal step of cell wall arabinan biosynthesis in Corynebacteriaceae, such as *Corynebacterium glutamicum* and *Mycobacterium tuberculosis*. *Journal of Biological Chemistry* **282**, 14729–14740.
- Senior, S. J., Illarionov, P. A., Gurcha, S. S., Campbell, I. B., Schaeffer, M. L., Minnikin, D. E. and Besra, G. S. (2003). Biphenyl-based analogues of thiolactomycin, active against *Mycobacterium tuberculosis* mtFabH fatty acid condensing enzyme. *Bioorganic and Medicinal Chemistry Letters* **13**, 3685–3688.
- Senior, S. J., Illarionov, P. A., Gurcha, S. S., Campbell, I. B., Schaeffer, M. L., Minnikin, D. E. and Besra, G. S. (2004). Acetylene-based analogues of thiolactomycin, active against *Mycobacterium tuberculosis* mtFabH fatty acid condensing enzyme. *Bioorganic and Medicinal Chemistry Letters* **14**, 373–376.
- Sham, L. T., Butler, E. K., Lebar, M. D., Kahne, D., Bernhardt, T. G. and Ruiz, N. (2014). Bacterial cell wall. MurJ is the flippase of lipid-linked precursors for peptidoglycan biogenesis. *Science* **345**, 220–222.
- Shi, L., Berg, S., Lee, A., Spencer, J. S., Zhang, J., Vissa, V., McNeil, M. R., Khoo, K. H. and Chatterjee, D. (2006). The carboxy terminus of EmbC from *Mycobacterium smegmatis* mediates chain length extension of the arabinan in lipoarabinomannan. *Journal of Biological Chemistry* **281**, 19512–19526.
- Shilpi, J. A., Ali, M. T., Saha, S., Hasan, S., Gray, A. I. and Seidel, V. (2015). Molecular docking studies on InhA, MabA and PanK enzymes from *Mycobacterium tuberculosis* of ellagic acid derivatives from *Ludwigia adscendens* and *Trewia nudiflora*. *In Silico Pharmacology* **3**, 10.
- Sink, R., Sosic, I., Zivec, M., Fernandez-Menendez, R., Turk, S., Pajk, S., Alvarez-Gomez, D., Lopez-Roman, E. M., Gonzales-Cortez, C., Rullas-Triconado, J., Angulo-Barturen, I., Barros, D., Ballell-Pages, L., Young, R. J., Encinas, L. and Gobec, S. (2015). Design, synthesis, and evaluation of new thiadiazole-based direct inhibitors of enoyl acyl carrier protein reductase (InhA) for the treatment of tuberculosis. *Journal of Medicinal Chemistry* **58**, 613–624.
- Sipos, A., Pato, J., Szekely, R., Hartkoorn, R. C., Kekesi, L., Orfi, L., Szantai-Kis, C., Mikusova, K., Svetlikova, Z., Kordulakova, J., Nagaraja, V., Godbole, A. A., Bush, N., Collin, F., Maxwell, A., Cole, S. T. and Keri, G. (2015). Lead selection and characterization of antitubercular compounds using the Nested Chemical Library. *Tuberculosis (Edinburgh)* **95** (Suppl. 1), S200–S206.
- Siricilla, S., Mitachi, K., Wan, B., Franzblau, S. G. and Kurosu, M. (2015). Discovery of a capuramycin analog that kills nonreplicating *Mycobacterium tuberculosis* and its synergistic effects with translocase I inhibitors. *Journal of Antibiotics* **68**, 271–278.
- Skovierova, H., Larrouy-Maumus, G., Zhang, J., Kaur, D., Barilone, N., Kordulakova, J., Gilleron, M., Guadagnini, S., Belanova, M., Prevost, M. C., Gicquel, B., Puzo, G., Chatterjee, D., Brennan, P. J., Nigou, J. and Jackson, M. (2009). AftD, a novel essential arabinofuranosyltransferase from mycobacteria. *Glycobiology* **19**, 1235–1247.
- Skovierova, H., Larrouy-Maumus, G., Pham, H., Belanova, M., Barilone, N., Dasgupta, A., Mikusova, K., Gicquel, B., Gilleron, M., Brennan, P. J., Puzo, G., Nigou, J. and Jackson, M. (2010). Biosynthetic origin of the galactosamine substituent of Arabinogalactan in *Mycobacterium tuberculosis*. *Journal of Biological Chemistry* **285**, 41348–41355.
- Stanley, S. A., Grant, S. S., Kawate, T., Iwase, N., Shimizu, M., Wivagg, C., Silvis, M., Kazyanskaya, E., Aquadro, J., Golas, A., Fitzgerald, M., Dai, H., Zhang, L. and Hung, D. T. (2012). Identification of novel inhibitors of *M. tuberculosis* growth using whole cell based high-throughput screening. *ACS Chemical Biology* **7**, 1377–1384.
- Stanley, S. A., Kawate, T., Iwase, N., Shimizu, M., Clatworthy, A. E., Kazyanskaya, E., Sacchettini, J. C., Ioerger, T. R., Siddiqui, N. A., Minami, S., Aquadro, J. A., Grant, S. S., Rubin, E. J. and Hung, D. T. (2013). Diarylcoumarins inhibit mycolic acid biosynthesis and kill *Mycobacterium tuberculosis* by targeting FadD32. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 11565–11570.
- Tahlan, K., Wilson, R., Kastrinsky, D. B., Arora, K., Nair, V., Fischer, E., Barnes, S. W., Walker, J. R., Alland, D., Barry, C. E., III and Boshoff, H. I. (2012). SQ109 targets MmpL3, a membrane transporter of trehalose monomycolate involved in mycolic acid donation to the cell wall core of *Mycobacterium tuberculosis*. *Antimicrobial Agents and Chemotherapy* **56**, 1797–1809.
- Takayama, K., Wang, C. and Besra, G. S. (2005). Pathway to synthesis and processing of mycolic acids in *Mycobacterium tuberculosis*. *Clinical Microbiology Reviews* **18**, 81–101.
- Tam, P. H. and Lowary, T. L. (2010). Epimeric and amino disaccharide analogs as probes of an alpha-(1→6)-mannosyltransferase involved in mycobacterial lipoarabinomannan biosynthesis. *Organic & Biomolecular Chemistry* **8**, 181–192.
- Thanna, S., Knudson, S. E., Grzegorzewicz, A., Kapil, S., Goins, C. M., Ronning, D. R., Jackson, M., Slayden, R. A. and Sucheck, S. J. (2016). Synthesis and evaluation of new 2-aminothiophenes against *Mycobacterium tuberculosis*. *Organic & Biomolecular Chemistry* **14**, 6119–6133.
- Tomasic, T., Zidar, N., Kovac, A., Turk, S., Simcic, M., Blanot, D., Muller-Premru, M., Filipic, M., Grdadolnik, S. G., Zega, A., Anderluh, M., Gobec, S., Kikelj, D. and Peterlin Masic, L. (2010). 5-Benzylidenethiazolidin-4-ones as multitarget inhibitors of bacterial Mur ligases. *ChemMedChem* **5**, 286–295.
- Tran, A. T., Wen, D., West, N. P., Baker, E. N., Britton, W. J. and Payne, R. J. (2013). Inhibition studies on *Mycobacterium tuberculosis* N-acetylglucosamine-1-phosphate uridylyltransferase (GlmU). *Organic & Biomolecular Chemistry* **11**, 8113–8126.
- Trivedi, O. A., Arora, P., Sridharan, V., Tickoo, R., Mohanty, D. and Gokhale, R. S. (2004). Enzymic activation and transfer of fatty acids as acyl-adenylates in mycobacteria. *Nature* **428**, 441–445.
- Trunkfield, A. E., Gurcha, S. S., Besra, G. S. and Bugg, T. D. (2010). Inhibition of *Escherichia coli* glycosyltransferase MurG and *Mycobacterium tuberculosis* Gal transferase by uridine-linked transition state mimics. *Bioorganic and Medicinal Chemistry* **18**, 2651–2663.
- Turnbull, W. B., Shimizu, K. H., Chatterjee, D., Homans, S. W. and Treumann, A. (2004). Identification of the 5-methylthiopentose substituent in *Mycobacterium tuberculosis* lipoarabinomannan. *Angewandte Chemie, International Edition In English* **43**, 3918–3922.
- van Heijenoort, Y., Leduc, M., Singer, H. and van Heijenoort, J. (1987). Effects of moenomycin on *Escherichia coli*. *Journal of General Microbiology* **133**, 667–674.
- Varela, C., Rittmann, D., Singh, A., Krumbach, K., Bhatt, K., Eggeling, L., Besra, G. S. and Bhatt, A. (2012). MmpL genes are associated with mycolic acid metabolism in mycobacteria and corynebacteria. *Chemistry and Biology* **19**, 498–506.
- Vilcheze, C., Baughn, A. D., Tufariello, J., Leung, L. W., Kuo, M., Basler, C. F., Alland, D., Sacchettini, J. C., Freundlich, J. S. and Jacobs, W. R., Jr. (2011). Novel inhibitors of InhA efficiently kill *Mycobacterium tuberculosis* under aerobic and anaerobic conditions. *Antimicrobial Agents and Chemotherapy* **55**, 3889–3898.
- Vollmer, W., Blanot, D. and de Pedro, M. A. (2008). Peptidoglycan structure and architecture. *FEMS Microbiology Reviews* **32**, 149–167.
- Wang, L. Q., Falany, C. N. and James, M. O. (2004). Triclosan as a substrate and inhibitor of 3'-phosphoadenosine 5'-phosphosulfate-sulfotransferase and UDP-glucuronosyl transferase in human liver fractions. *Drug Metabolism and Disposition: The Biological Fate of Chemicals* **32**, 1162–1169.
- Warrier, T., Tropis, M., Werngren, J., Diehl, A., Gengenbacher, M., Schlegel, B., Schade, M., Oschkinat, H., Daffe, M., Hoffner, S., Eddine, A. N. and Kaufmann, S. H. (2012). Antigen 85C inhibition restricts *Mycobacterium tuberculosis* growth through disruption of cord factor biosynthesis. *Antimicrobial Agents and Chemotherapy* **56**, 1735–1743.
- Watanabe, M., Aoyagi, Y., Ridell, M. and Minnikin, D. E. (2001). Separation and characterization of individual mycolic acids in representative mycobacteria. *Microbiology* **147**, 1825–1837.
- Watanabe, M., Aoyagi, Y., Mitome, H., Fujita, T., Naoki, H., Ridell, M. and Minnikin, D. E. (2002). Location of functional groups in mycobacterial meromycolate chains; the recognition of new structural principles in mycolic acids. *Microbiology* **148**, 1881–1902.
- Wiedemann, I., Breukink, E., van Kraaij, C., Kuipers, O. P., Bierbaum, G., de Kruijff, B. and Sahl, H. G. (2001). Specific binding of nisin to the peptidoglycan precursor lipid II combines pore formation and inhibition of cell wall biosynthesis for potent antibiotic activity. *Journal of Biological Chemistry* **276**, 1772–1779.
- Wilson, R., Kumar, P., Parashar, V., Vilcheze, C., Veyron-Churlet, R., Freundlich, J. S., Barnes, S. W., Walker, J. R., Szymonifka, M. J., Marchiano, E., Shenai, S., Colangeli, R., Jacobs, W. R., Jr., Neiditch, M. B., Kremer, L. and Alland, D. (2013). Antituberculosis thiophenes define a requirement for Pks13 in mycolic acid biosynthesis. *Nature Chemical Biology* **9**, 499–506.

- Wolucka, B. A., McNeil, M. R., de Hoffmann, E., Chojnacki, T. and Brennan, P. J.** (1994). Recognition of the lipid intermediate for arabinogalactan/arabinomannan biosynthesis and its relation to the mode of action of ethambutol on mycobacteria. *Journal of Biological Chemistry* **269**, 23328–23335.
- World Health Organization** (2014). *Global Tuberculosis Report*. World Health Organisation, Geneva, Switzerland.
- World Health Organization** (2016). *Global Tuberculosis Report*. World Health Organisation, Geneva, Switzerland.
- Zhang, J., Angala, S. K., Pramanik, P. K., Li, K., Crick, D. C., Liav, A., Jozwiak, A., Swiezewska, E., Jackson, M. and Chatterjee, D.** (2011). Reconstitution of functional mycobacterial arabinosyltransferase AftC proteoliposome and assessment of decaprenylphosphorylarabinose analogues as arabinofuranosyl donors. *ACS Chemical Biology* **6**, 819–828.
- Zhang, Y. and Young, D.** (1994). Molecular genetics of drug resistance in *Mycobacterium tuberculosis*. *Journal of Antimicrobial Chemotherapy* **34**, 313–319.
- Zhang, Z., Bulloch, E. M., Bunker, R. D., Baker, E. N. and Squire, C. J.** (2009). Structure and function of GlmU from *Mycobacterium tuberculosis*. *Acta Crystallographica. Section D: Biological Crystallography* **65**, 275–283.